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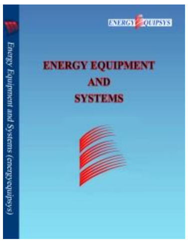

Investigation and comparison of bench-scale anaerobic digestion system performance under thermophilic and mesophilic conditions for producing biomethane from solid organic wastes

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ABSTRACT

The outcomes of every batch of anaerobic digesters vary due to differences in equipment, feed composition, and operating conditions. The performance of a bench-scale anaerobic digestion system was investigated in this study with the goal of biomethane production, considering mesophilic requirements at 37°C and thermophilic conditions at 57°C. Three experiments were carried out under mesophilic and thermophilic conditions. A blend of water and solid organic waste was used in the first experiment. The second test involved a mixture of water, material that had undergone prior digestion, and solid organic waste. The third trial incorporated water, previously digested material, solid organic waste, and cow manure as part of the feed. Based on the result, in the third experiment, over 32 days within mesophilic conditions, the average daily biogas and biomethane production per unit of feed mass was quantified as 1610 and 447.10 ml, respectively. Furthermore, the measurements indicated 62.22% and 99.99% increases compared to similar feed subjected to thermophilic conditions. Moreover, the data displayed growth rates of 8.05%, 96.04%, and a substantial rise of 69.47% and 147.7% compared to the outcomes of the second and third experiments performed under mesophilic conditions. Additionally, it can be included that the digestion period of the third test was 60 days shorter than that of the second and one day less than the first trial when operating under mesophilic conditions. As a result, the most effective method for anaerobic digestion involved blending solid organic waste, previously digested material, cow manure, and water under mesophilic conditions.

Keywords: Biogas, Hydrolysis, Acidolysis, Acetogenic, Methanogenic.

1.Introduction

Anaerobic digestion (AD) is influenced by several critical factors, including temperature range, carbon-to-nitrogen ratio, pH levels, organic loading rate, and solid retention time. Among these, the operational temperature significantly impacts the AD process [1]. Achieving alignment between the operating temperature, the type of anaerobic digester, and other equipment is crucial for attaining the desired outcomes. Investigations have been

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carried out to explore the consequences of applying mesophilic (M) conditions (within the range of 35 to 40 °C) and thermophilic (T) conditions (ranging from 55 to 60 °C) to the AD process involving different organic materials. Considering the conclusions from multiple prior research, it is evident that while T conditions yield a substantial biogas output surpassing M conditions, the methane content remains modest. Sutaryo et al. [2] proved that biomethane's low content is due to hydrogen escaping to the gas phase and the inaccessibility of methanogenic microorganisms to hydrogen molecules to produce methane at T conditions. Also, the ammonia inhibitory effect on methanogenic microorganisms' activity in the T process is greater than that of M. In other research, Ferrer et al. [3] demonstrated that produced useful energy from sludge AD in T conditions is competitive with M in half residence time. Moreover, Riggio et al. [4] declared that AD of cow manure (CM) in batch leach–bed reactors at T conditions resulted in rapid feed decomposition and had the same efficiency compared to M during 42 days.

Moreover, in batch leach–bed reactors, the AD of cow manures (CM) under T conditions prompts rapid breakdown of the feed material, showcasing comparable efficiency to M conditions over a 42-day duration [5]. Another research revealed that the AD of high-fiber cattle manure exhibited a greater methane output when subjected to T conditions compared to M conditions, as evidenced by two anaerobic reactors operating at laboratory and pilot scales [6]. On the other hand, mesophilic reactors demonstrated elevated biogas generation and enhanced performance stability compared to T reactors, mainly when dealing with high organic load rates during the extended AD process for food waste. Based on a study, over a 110-day digestion period, acetoclastic methanogenic microorganisms exhibited heightened activity. The oxidation of propionate and butyrate was more noticeable under M conditions than under T. Additionally, the development of calcium-containing crystals, which moderate the inhibitory impact of long-chain fatty acids in the AD process, demonstrated superior performance under M conditions compared to T [7]. Research findings indicate that the efficiency and stability of methane production from food waste digestion are superior under M conditions compared to T states. This discrepancy is attributed to the accumulation of free fatty acids and the inhibitory impact of generated ammonia in T conditions [2, 8].

The majority of research shows that biomass AD is more efficient in M than T environments; however, some research contradicts this. Selecting the best temperature conditions, whether M or T, for an AD process, is determined by factors like feed composition, the kind of digester used, other equipment within the digestion unit, and the intended purpose of the process. Hence, studying how each AD setup behaves with various feeds or targets can produce unique results at different operating temperatures. The primary aim of this investigation was to explore and obtain a comparison regarding the performance of a bench-scale AD setup customized for the treatment of solid organic wastes (SOW). This analysis was conducted under both M and T conditions, particularly with the goal of biomethane production.

2. Materials and methods

2.1. Equipment

The configuration of devices employed in this study is presented in Fig. 1. The central portion of the digestion tank was constructed with a double-wall design to facilitate heating. It was also insulated using a glass wool layer to minimize heat loss. For fluid heating, a bain-marie water bath (Memmert D91126) was utilized. In order to thoroughly blend the internal contents of the digester, a stainless steel anchor stirrer was employed (Fig. 2) [9]. The stirrer blade maintained a distance of 2 cm from the base and walls of the digester tank, thereby being powered by a DC power supplier (GWINSTEK GPS-3303C), which supplies the necessary current intensity and voltage for rotation. The temperature of the substances inside the digester was measured using a thermocouple, and the resulting temperature reading was shown on a screen with an accuracy of ±0.1 °C. Moreover, the gauge pressure (1953 WINTERS) was utilized to measure intra-digester pressure and the produced biogas. The generation of anaerobic conditions and removing air/produced biogas from the system at the end or throughout the procedure were achieved using a vacuum pump (PLATINUM JB 60507). The vacuum pump outlet was connected to a Bunsen burner for flame testing. Gas phase analysis and associated examinations involved using two tanks in the system: one with a capacity of 13 ml and another larger tank with a volume of 2 liters. The release of the biogas produced by the system was channeled through a connected pathway to a safety valve and further into a polyethylene tank with a volume of 250 liters.

2.2. Material

Primarily, the main component required is the feed. The feed comprised a mixture of degradable organic substances, including SOW, material previously subjected to digestion in an earlier stage (PSD), and CM.

2.3. Experimental method

Three tests were calibrated and implemented for both the M and T phases. The feed for the initial to third tests in both sets was composed of the following: a combination of crushed SOW and water; a mixture of crushed SOW, material digested in the first stage, and water; and a blend of crushed SOW, material digested in the second stage, CM, and water. These selections were made with equal weight ratios. For each assessment, a portion of 200 g from the feed mixture was assigned for experimental purposes, while the remaining portion was introduced into the digester.

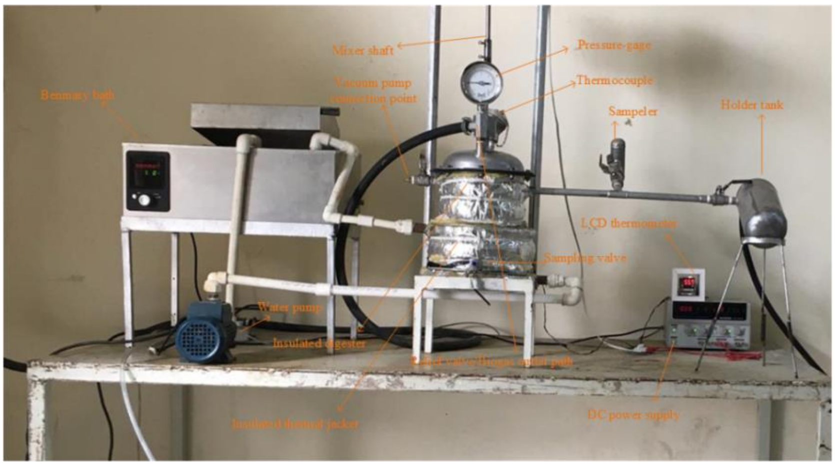

Fig. 1. AD system set

Fig. 2. An Anchor stirrer was installed inside the digester

To establish anaerobic conditions, a vacuum pump was employed, subjecting the system to a relative pressure of -0.75 barg after sealing the digester cap. Mesophilic and thermophilic conditions were established through hot water circulation at 37 and 57 °C, respectively. This was achieved using a heat jacket within the digester for M conditions and a bain-marie water bath for T conditions. The substrate was uniformly mixed by a mechanical stirrer for 3 hours, three days per week, operating at an average rotational speed of 10 rpm [10].

The biochemical process of AD occurred gradually alongside the generation of biogas. Throughout the digestion process, the system's pressure experienced a rise attributable to biogas production. Subsequently, this pressure decreased and eventually reached a stable state following the attainment of a peak. Throughout this period, an ongoing process of measurement and recording was carried out to track the temperature, pressure, and volume of biogas produced. The system's thermocouples, barometers, and volumetric equipment facilitated the data collection. At the end of the procedure, the sampler was detached from the system to facilitate the analysis of biogas. The criterion for signaling the end of the process was maintaining a minimal relative pressure of the biogas produced by the system, lasting for one week after attaining the highest peak. Subsequently, the biogas contained within the system was released and directed into a 250-liter storage tank via the outlet pathway of the safety valve, facilitated by a vacuum pump. Following this step, the cap of the digester was opened, and a sample of 200 g of digested material was extracted for analysis. Eventually, the entire digester’s contents were evacuated, preparing the system for further examinations.

2.4. Samples Analysis Method

The analyses were categorized into two primary groups: physical chemistry and instrumental analyses. These analyses involved determining and measuring the feed relative composition, digested products, and biogas. In the context of physical chemistry, specific parameters were evaluated, encompassing percentages related to moisture, dry elements, ash content, and organic constituents. The assessment of these percentages corresponded with the established national standards of Iran as outlined in references [11, 12, 13]. The elemental composition of feed and digested products, encompassing elements such as carbon, nitrogen, hydrogen, and sulfur, was determined using an elemental analyzer (Costech instrumental elemental combustion system model and Eager 300 model for EA1112). Furthermore, the relative composition of the biogas was assessed utilizing gas chromatography (GC), facilitated by the use of the HP-PLOT Q column (DG1DCE40DE).

3. Results and discussion

The elevation in the percentage of digested moisture composition correlates directly with the heightened generation of biogas and biomethane, which can be attributed to the activity of methanogenic microorganisms [14]. That is,

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (1)$$

Figure 3 illustrates the changes in the proportion of digested moisture compared to the feed across all three experiments conducted during the M and T experimental phases. The conversion percentage of moisture from the first to the third test has experienced a rise, pointing to increased engagement of methanogenic microorganisms and a proportionally more significant presence of methane in the set of M conditions. Conversely, the moisture conversion percentage in the T condition not only declined compared to the M condition, but also a decreasing trend was observed in the entire test under T conditions. These fluctuations arise from the diminished activity of methanogenic microorganisms in T conditions [7, 15]. These deviations validate the suppression of active microorganisms within CM and PSD under T conditions.

Nonetheless, the increase in free fatty acid concentration, resulting from the hydrolysis process induced by the elevated temperature of T conditions, prevents biogas production and leads to a rise in moisture content [16, 17].

The involvement of microorganisms in biochemical processes facilitates the conversion of organic and volatile fractions into biogas [18]. Consequently, reducing the fraction of digested material relative to the feed demonstrates the proportionate generation of biogas. Therefore, reducing this constituent in the digested material compared to the feed will be directly proportional to the volume of biogas generated.

Figure 4 illustrates the percentage conversion of the organic portion within the digested material compared to the feed for each test conducted under both M and T conditions. In the M state, which involves the response of acetogenic and methanogenic microorganisms to the prevalent process temperature, organic acid compounds formed through hydrolysis and acidolysis underwent a conversion process that generates biogas. Notably, this environment possibly prevents the accumulation of volatile organic acids, enabling the sequential progression of AD process reactions [14, 19]. Furthermore, during the second and third examinations conducted under M conditions, the active participation of microorganisms in PSD and CM and their alignment with the process requirements accelerate these mechanisms. Notably, the initial phases of hydrolysis and acidolysis in the first trial carried out under the elevated temperatures of T conditions, displayed pronounced advancement and remarkable progress compared to similar situations in the M state. Nonetheless, in the second and third trials conducted under T conditions, the percentage of organic part conversion compared to analogous M conditions and between themselves experienced a reduction. This reduction is attributed to the heightened accumulation of volatile fatty acids from hydrolysis and acidolysis. Significantly, this accumulation inhibits the progression of the third and fourth stages of AD reactions due to the inadequate adaptation of acetogenic and methanogenic microorganisms [20].

Figures 5 and 6 illustrate the conversion rates of carbon and nitrogen relative compositions within the digested material compared to the feed in each test conducted under M and T conditions. The carbon and nitrogen conversion percentages observed in the M tests reflected an increase within the digested material when contrasted with the feed. Conversely, within T conditions, these shifts exhibited a decrease. It is worth mentioning that, in the first test, these changes occurred faster in T conditions than in M, whereas in the second and third tests, changes occurred slower than in M (Figs. 3 and 4). Moreover, under T conditions, a rise in the generation of ammonia (NH_3) and ammonium ions (NH_4^+) occurred due to the hydrolysis and acidolysis of nitrogen-rich compounds, such as proteins. This heightened production of NH_3 and NH_4^+ contributes to an inhibitory influence on the biomethane production mechanism, thereby affecting the activity of methanogenic microorganisms. Consequently, this phenomenon leads to a notable decline in the overall biogas output, particularly in biomethane synthesis [21, 22, 23, 24].

Figures 7-9 demonstrate a comparative analysis of the daily biogas and biomethane production volumes per unit mass of feed in experiments conducted under M and T conditions. Based on the findings, it is evident that the daily rate of biogas production per unit mass of feed was consistently lower in all tests carried out under T conditions compared to M. This difference became notably pronounced from the first to third tests, respectively.

The biomethane volume generation rate exhibited a corresponding behavior: the biomethane output from the initial and second tests under T conditions was exceedingly minimal, nearly negligible, and in the third test, it remained markedly lower compared to the output observed under M conditions.

Increasing process temperatures accelerated complex compounds' hydrolysis and acidolysis phases, mainly cellulose. This phenomenon was notable primarily in the initial tests when the feed consisted solely of a blend of SOW and water. However, these enhanced temperatures prevented the progression of acetogenic and methanogenic stages, particularly evident in the second and third tests. Consequently, this impediment led to a reduction in the volume of biogas generated.

This phenomenon arises from volatile fatty acids accumulating, which are succeeded by hydrolysis and acidolysis. It is further influenced by the inhibition of nitrogenous compounds (ammonia and ammonium) and the hydrolysis and acidolysis of proteins. Additionally, the escape of hydrogen molecules and the limited accessibility of methanogenic microorganisms to these molecules due to the elevated temperature characteristic of the T process collectively contribute to a marked reduction in the volume of biomethane produced across all three tests [14].

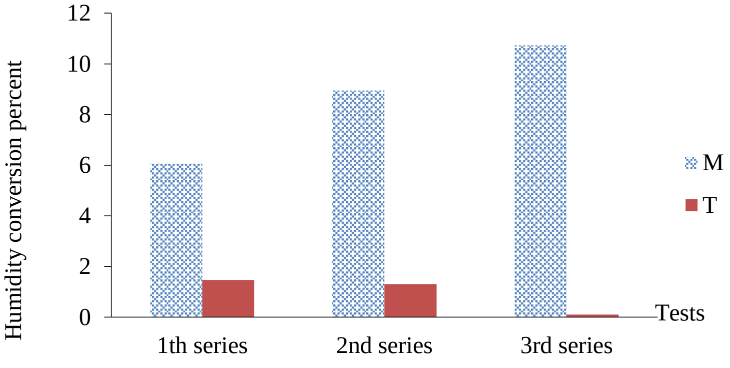

Fig. 3. Conversion percentage of digested material moisture compared with feed

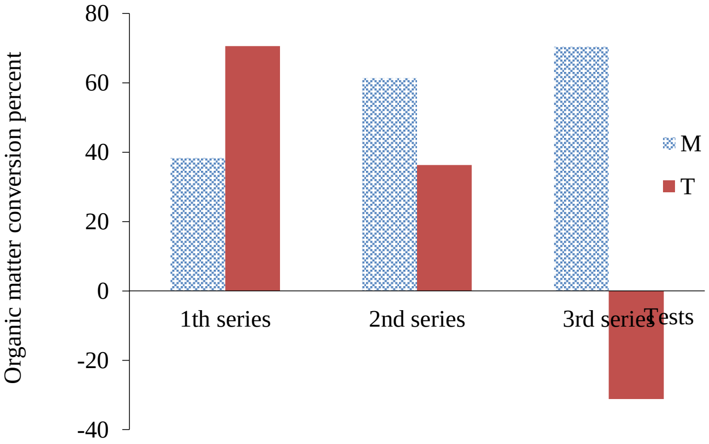

Fig. 4. Organic part conversion percentage of digested material compared with feed

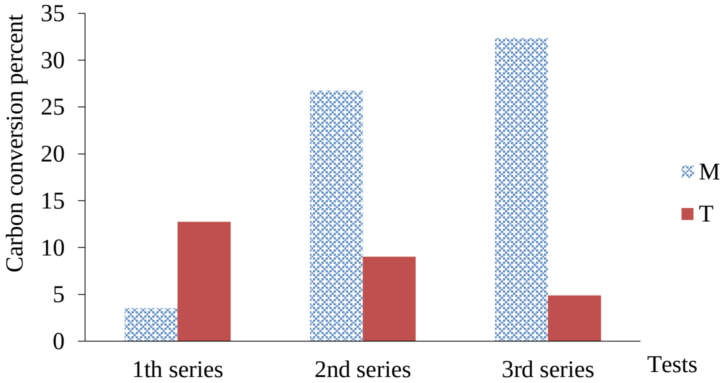

Fig. 5. Carbon conversion percent in digested material compared with feed

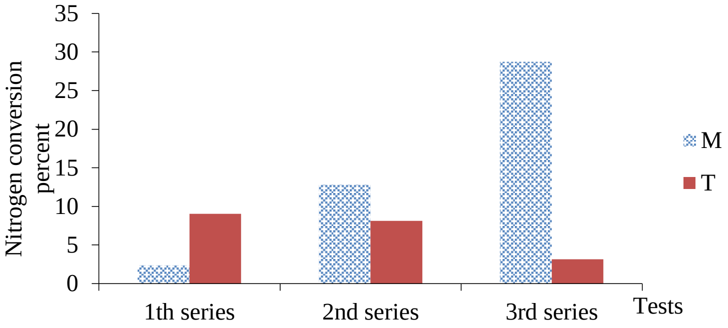

Fig. 6. Nitrogen conversion percent in digested material compared with feed

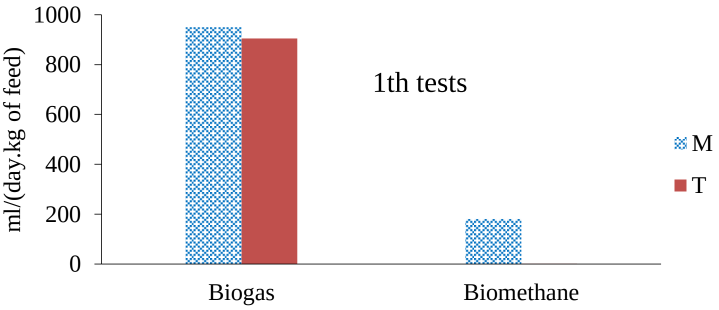

Fig. 7. Comparison of produced biogas and biomethane daily volume rate per feed mass unit of first tests under M and T conditions

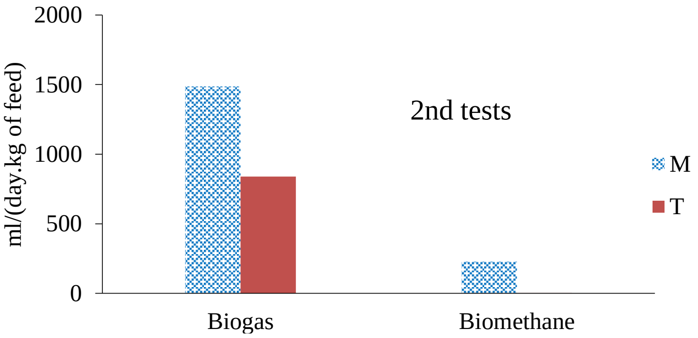

Fig. 8. Comparison of produced biogas and biomethane daily volume rate per feed mass unit of second tests under M and T conditions

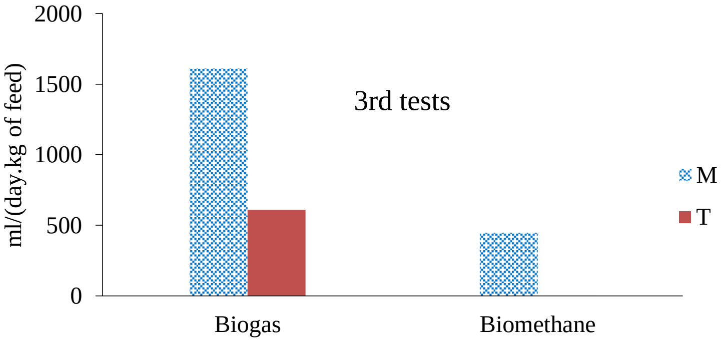

Fig. 9. Comparison of produced biogas and biomethane daily volume rate per feed mass unit of third tests under M and T conditions

Table 1. Gas chromatography analysis (GC) results of accumulated biogas during the AD period

Biogas Composition percent	1th tests		2nd tests		3rd tests	
	M	T	M	T	M	T
CO_2	80.41	91.58	69.27	92.33	66.8	93.73
CO	19.4	8.42	15.37	7.67	5.42	6.26
CH_4	0.19	0.0010	15.35	0.0015	27.77	0.0052

Table 1 presents the analyzed findings regarding the cumulative biogas generated throughout the AD duration, encompassing methane, carbon dioxide, and carbon monoxide, for each test conducted under M and T conditions. In light of the outcomes derived from the M experiments, there was a consecutive decrease in the relative proportion of carbon dioxide from the first to the third tests while simultaneously observing a corresponding increase in the relative proportion of methane.

This process is attributed to the presence and successful adaptation of active methanogenic microorganisms to the environmental conditions in the second and third tests, facilitated by the co-digestion of SOW alongside PSD and/or CM. Meanwhile, the relative proportion of methane remains relatively low in the T experiments. Consequently, the co-digestion of SOW with PSD and/or CM has a limited impact on altering these compositional percentages. These results originate from multiple factors discussed in earlier sections, primarily centering around the discrepancy between active methanogenic microorganisms and the conditions characteristic of the T process.

Finally, Figs. 10-12 provide a comparative analysis of the relative pressure fluctuations of daily biogas production per unit mass of feed in each test conducted under M and T conditions throughout the AD period. As depicted in Fig. 10, the pressure fluctuations in biogas production started from -0.175 bar/kg under M conditions and -0.123 bar/kg under T conditions. These fluctuations gradually increased following the progression of AD and biogas generation. Within the M process, the pressure reached 0.013 bar/kg over a duration of 10 days, subsequently stabilizing at a consistent level of 0.025 barg/kg fluctuation from the 33rd day onwards. During the T process, the system pressure peaked at 0.023 bar/kg within a day and then stabilized at 0.015 bar/kg following fluctuations that persisted for 24 days. Significantly, the heightened temperature in T conditions accelerated the hydrolysis and acidolysis processes. The end result of these stages led to the formation of volatile fatty acids, water, and carbon dioxide. However, on the other hand, the diminished or delayed activity of acetogenic and methanogenic microorganisms has also led to the accumulation of volatile fatty acids, thus preventing methane production and effectively stopping the advancement of hydrolysis and acidolysis processes.

Conversely, the deceleration of hydrolysis and acidolysis reactions, attributed to the lower temperature prevalent in the M stage, is coupled with the delayed onset and inactive performance of acetogenic and methanogenic microorganisms in the solitary AD procedure for SOW. This combination led to the alterations illustrated in Fig, 10. According to Fig. 11, the fluctuations in biogas pressure throughout the M process initiated at -0.199 bar/kg on the initial day. Over the subsequent 38-day duration, a progressive elevation was terminated in a 0.222 bar/kg value. Following this, the pressure steadied, experiencing irregular fluctuations on the 92nd day at approximately 0.035 bar/kg, ultimately signifying the termination of the process.

In contrast, the pressure variations in the T process initiated at -0.93 bar/kg. They reached a peak value of 0.044 bar/kg on the second day and then gradually settled at 0.025 bar/kg after several fluctuations spanning 62 days. This ultimately led to the termination of the process.

Moreover, alongside the explanations well-found for interpreting the T diagram presented in Fig. 10, which can also be extended to Fig. 11, it becomes evident that the presence of active microorganisms in PSD does not enhance the AD process when subjected to T conditions. The heightened temperature restricts the adaptability of these microorganisms, ultimately leading to their deactivation. In contrast, a gradual noticeable enhancement becomes evident under M conditions due to the co-digestion of SOW with PSD, demonstrating a progressive tendency towards completing four consecutive stages of AD. The capability of dynamic microorganisms to adjust to the process parameters results in a rise in biogas pressure, subsequently providing the potential for an increase in methane production [25]. As depicted in Fig. 12, fluctuations in biogas pressure for both M and T conditions originated from an initial point of -0.102 bar/kg. Within 5 days, the highest pressure values recorded for M and T conditions were 0.14 and 0.048 barg/kg, respectively.

Afterward, in both cases, following fluctuations on the 32nd day, the AD process was finalized by stabilizing at a consistent pressure of 0.023 bar/kg. The explanations offered to interpret the shifts in Figs. 10 and 11 concerning the patterns depicted in Fig. 12 are also valid and applicable. Notably, the duration of the AD process is reduced in M conditions, aligning with the observed timeframe in T conditions.

In the M condition, small amounts of trace elements (Cobalt, Nickel, Copper, Manganese, Iron, Zinc, Selenium, and Molybdenum) that are present in CM, along with the active microorganisms essential for the four stages of AD within CM and PSD, play a vital role in accelerating the process. On the other hand, despite the presence of these elements, their ability to induce the quadruple AD stages is limited by the challenge of microorganisms adapting to the elevated temperatures associated with T conditions.

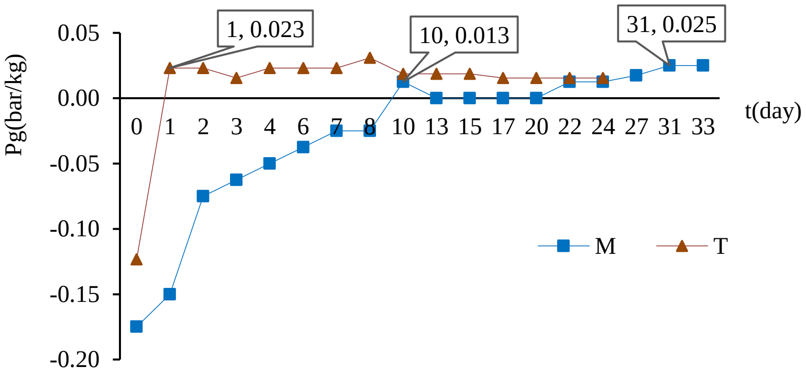

Fig. 10. The pressure of produced biogas per feed mass unit of first tests during the AD process under M and T conditions.

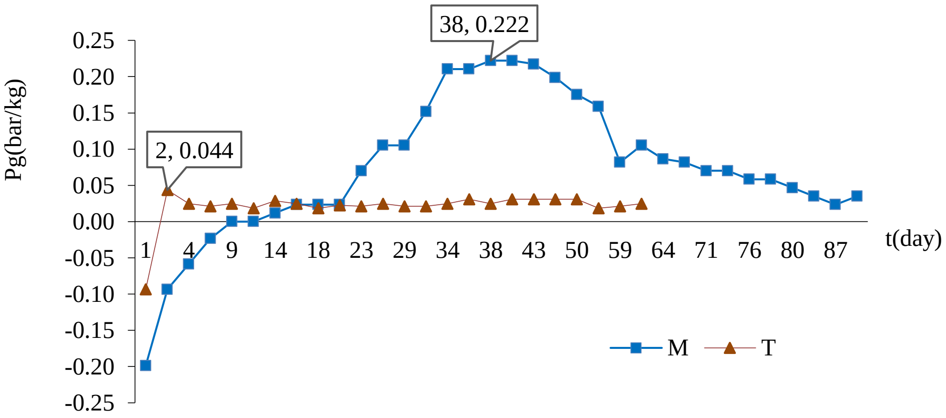

Fig. 11. The pressure of produced biogas per feed mass unit of second tests during the AD process under M and T conditions.

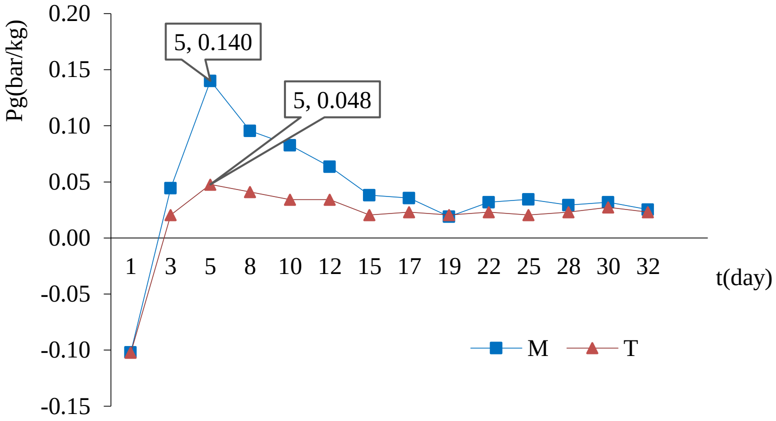

Fig. 12. The pressure of produced biogas per feed mass unit of M and T third tests during the AD process.

4. Conclusion

This study investigated the performance of a bench-scale anaerobic digester apparatus set under T and M conditions. Three different feed compositions were used: a mixture of SOW and water; a mixture of SOW, water, and PSD; and a mixture of SOW, water, PSD, and CM. The main aim was to assess biomethane production. The findings from the experiments revealed that the most optimal performance was achieved through the co-digestion of the SOW, water, PSD, and CM mixture at 37°C (M conditions). In this case, over 32 days, a daily biogas yield of 1610 ml was achieved per unit mass of the provided feed, incorporating 447.10 ml of biomethane. These results were compared with the output from similar feed subjected to T conditions at 57 °C within the same digestion timeframe.

In these conditions, a significant increase was observed in the generated biogas and biomethane volumes, amounting to 62.11% and 99.99%, respectively, compared to the outcomes attained under T conditions. Additionally, an analysis of the performance outcomes across all three feed types within M conditions revealed the following: The third test displayed a growth of 8.05% and 96.04% in biogas and biomethane production compared to the second test and an increase of 69.47% and 147.7% in comparison to the first. Notably, the digestion period for the third test decreased by 60 days compared to the second test and by a single day compared to the first.

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References

[1] Aydram, R., Haj Agha Alizade, H., Rasouli, M., Shadidi, B., Simplex Centroid Mixture Design for Optimizing and Promoting the Anaerobic Co-digestion Performance of Sheep Blood and Cheese Whey, Journal of Renewable Energy and Environment (2021) 8(3): 8-15. https://doi.org/10.30501/jree.2021.251583.1151

[2] Sutaryo, S., Ward, A. J., Moller, HB., Ammonia inhibition in thermophilic anaerobic digestion of dairy cattle manure, Journal of the Indonesian Tropical Animal Agriculture (2014) 39(2):83-90. https://doi.org/10.14710/jitaa.39.2.83-90

[3] Ferrer, I., Vázquez, F., Font, X., Comparison of the Mesophilic and Thermophilic Anaerobic Sludge Digestion from an Energy Perspective, Journal of Residuals Science & Technology (2011) 8(2): 81-87.

[4] Riggio, S., Hernandéz-Shek, MA., Torrijos, M., Vives, G., Esposito, G, van Hullebusch,

ED., Steyer, JP., Escudié, R., Comparison of the Mesophilic and Thermophilic Anaerobic Digestion of Spent Cow Bedding in Leach-bed Reactors, Bioresource Technology (2017) 234: 466-471. https://doi.org/10.1016/j.biortech.2017.02.056

[5] Moset, E., Poulsen, M., Wahid, R., Højberg, O., Møller, HB., Mesophilic Versus Thermophilic Anaerobic Digestion of Cattle Manure: Methane Productivity and Microbial Ecology, Microbial Biotechnology (2015) 8(5): 787–800. https://doi.org/10.1111/1751-7915.12271

[6] Guo, X., Kang, K., Shang, G., Yu, X., Qiu, L., Sun, G., Influence of Mesophilic and Thermophilic Conditions on the Anaerobic Digestion of Food Waste: Focus on the Microbial Activity and Removal of Long Chain Fatty Acids, Waste Management & Research (2018) 36(11):1106-1112. https://doi.org/10.1177%2F0734242X18801195

[7] Yirong, C., Banks, CJ., Heaven, S., Comparison of Mesophilic and Thermophilic Anaerobic Digestion of Food Waste, Faculty of Engineering and the Environment, University of Southampton, Southampton (2013), SO17 1BJ, UK.

[8] Yousefi, L., The Biogas Handbook (Science, Production and Application), Part Ⅱ: Plant Design, Engineering, Process Optimization and Digestate Utilization. (2021), Azad Academic Press, (in Farsi).

[9] Singh, B., Szamosi, Z., Siménfalvi, Z., State of the Art on Mixing in an Anaerobic Digester: A Review, Renewable Energy (2019) 141: 922-936. https://doi.org/10.1016/j.renene.2019.04.072.

[10] Anonymous., Compost – Physical and Chemical Specifications, Institute of Standards and Industrial Research of Iran (10716) (1991), 1st. edition, (in Farsi).

[11] Anonymous., Experimental Method for Determining Amount of Soil Moisture, Institute of Standards and Industrial Research of Iran (1677) (1991), 3st. edition, (in Farsi).

[12] Anonymous., Compost – Sampling and Physical and Chemical Tests Method, Institute of Standards and Industrial Research of Iran (13320) (1991), 1st. edition, (in Farsi).

[13] Yousefi, L., The Biogas Handbook (Science, Production and Application), Part 1: Biomass Resources, Feed Processing and Biogas Production. (2019), Atran Publications, (in Farsi).

[14] Palatsi, J., Laureni, M., Andrés, MV., Flotats, X., Nielsen, HB., Angelidaki, I., Strategies for Recovering Inhibition Caused by Long Chain Fatty Acids on Anaerobic Thermophilic Biogas Reactors, Bioresource Technology (2009) 100 (20): 4588 - 4596. https://doi.org/10.1016/j.biortech.2009.04.046)

[15] Nosrati, M., Shojaosadati, S., Sreekrishnan, T., Mukhopadhyay, S., Inhibition of Thermophilic Anaerobic Digestion of Waste Food by Long Chain Fatty Acids and Propionate, Iranian Journal of Biotechnology (2004) 2(4): 261-268. https://doi.org/10.1139/A10-011

[16] Palatsi, J., Illa, J., Prenafeta-Boldú, FX., Laureni, M., Fernandez, B., Angelidaki, I., Flotats, X., Long-chain Fatty Acids Inhibition and Adaptation Process in Anaerobic Thermophilic Digestion: Batch Tests, Microbial Community Structure and Mathematical Modelling, Bioresource Technology (2010) 101(7): 2243-2251. https://doi.org/10.1016/j.biortech.2009.11.069

[17] Yousefi, L, Effect of Mixing Digested with Municipal Solid Organic Waste in Biogas Production Through Anaerobic Digestion Bath System under Mesophilic Conditions, Journal of Green Chemistry and Sustainable Technologies (2020) 3: 61-69. http://gcst.ccerci.ac.ir/article_112889_b901878e9f9e3fe8b61ac6f2652f582f.pdf) (in Farsi).

[18] Zhang, W., Chen, B., Li, A., Zhang, L., Li, R., Yang, T., Xing, W., Mechanism of Process Imbalance of Long-term Anaerobic Digestion of Food Waste and Role of Trace Elements in Maintaining Anaerobic Process Stability, Bioresource Technology (2018) 275:172-182. https://doi.org/10.1016/j.biortech.2018.12.052

[19] Ma, J., Zhao, QB., Laurens, LLM., Jarvis, EE., Nagle, NJ., Chen, S., Frear, CS.,

Mechanism, Kinetics and Microbiology of Inhibition Caused by Long-chain Fatty Acids in Anaerobic Digestion of Algal Biomass, Biotechnol Biofuels, (2015) 8: 141. https://doi.org/10.1186/s13068-015-0322-z

[20] Yenigün, O., Burak Demirel, B., Ammonia Inhibition in Anaerobic Digestion: A Review, Process Biochemistry (2013) 48(5–6): 901-911. https://doi.org/10.1016/j.procbio.2013.04.012

[21] Shihwu Sung, S., Liu T., Ammonia Inhibition on Thermophilic Anaerobic Digestion, Chemosphere (2003) 53(1): 43-52. https://doi.org/10.1016/S0045-6535(03)00434-X

[22] Benabdallah El Hadj, T., Astals, S., Galí, A., Mace, S., Mata-Alvarez, J., Ammonia Influence in Anaerobic Digestion of OFMSW, Water Science Technology (2009) 59(6):1153-8. https://doi.org/10.2166/wst.2009.100

[23] Capson-Tojo, G., Moscoviz, R., Astals, S., Robles, Á., Steyer, JP., Unraveling the Literature Chaos Around Free Ammonia Inhibition in Anaerobic Digestion, Renewable and Sustainable Energy Reviews (2020) 117: 109487. https://doi.org/10.1016/j.rser.2019.109487

[24] Fischer, MA., Ulbricht, A., Neulinger, SC., Refai, S., Waßmann, K., Künzel, S., Schmitz, RA., Immediate Effects of Ammonia Shock on Transcription and Composition of a Biogas Reactor Microbiome, Frontiers in Microbiology Journal (2019) 10: 2064. https://doi.org/10.3389/fmicb.2019.02064

[25] Myszograj, S., Stadnik, A., Płuciennik-Koropczuk, E., The Influence of Trace Elements on Anaerobic Digestion Process, Civil and Environmental Engineering Reports Journal (2018) 28(4): 105-115. https://doi.org/10.2478/ceer-2018-0054