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Assessment of Methane Production Features and Kinetics from Poultry Dropping Waste under Mesophilic Conditions

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ABSTRACT

Waste from poultry droppings in Had Soualem is valuable because it is rich in organic molecules that break down easily. One way to use this waste is by making methane from it. This helps reduce its volume and its impact on the environment. We are studying how mixing this waste with green waste can assess the effect of co-digestion with a green waste co-substrate in batch mode, under mesophilic conditions at 37 °C and using an infinitely mixed laboratory digester continuous stirred tank reactor (CSTR) with a capacity of 1.5 liters. During this process, the parameters pH, TOC, NTK, COD, alkalinity, and conductivity at the digester were monitored at the laboratory scale, and volatile fatty acids were modified to promote the bioconversion of biomass into methane. When everything works just right, the reactor stays stable, and it can make up to 70% methane, with only 1 to 1.5% hydrogen sulfide. This shows that combining poultry waste with green waste could be a good way to deal with these types of organic waste, turning them into something valuable and making the process more appealing.

Keywords: methane production, features and kinetics, mesophilic anaerobic digestion, poultry dropping waste, Had Soualem.

INTRODUCTION

In recent years, there has been a steady increase in energy demand, primarily attributed to the growing global population. This demographic expansion has consequently elevated energy consumption across various sectors including industry, transportation, agriculture, and household needs (Ofoefule et al., 2009). Thus, it becomes imperative to explore sustainable and environmentally friendly solutions on a global scale. One promising strategy lies in harnessing the production of biogas through the anaerobic digestion of organic matter (Naimi et al., 2016). This technique offers the potential to convert diverse organic wastes such as food scraps, grass clippings, and septic tank effluents into usable energy. The recent research into producing biogas from diluted poultry droppings mixed with bleeding water from Had Soualem's slaughterhouse in Morocco has shown promising potential for converting this organic waste into energy. This method not only lessens the environmental impact of waste but also generates renewable energy (Alfa et al., 2014). Poultry droppings pose a significant challenge to environmental sustainability, as they are unavoidable residues from the poultry industry. They are rich in nutrients like phosphorus and nitrogen, making them a sustainable option for managing waste and producing renewable energy (Boughaba, 2011).

Anaerobic digestion (AD) is a process that involves treating organic waste in an environment with low oxygen levels inside an anaerobic digester. This method offers significant advantages in reducing greenhouse gas emissions

(Beniche et al., 2021). The AD process consists of four main stages: hydrolysis, fermentation, acetogenesis, and methanogenesis, resulting in the production of biogas primarily composed of methane (CH_4) and carbon dioxide (CO_2) (Patinvoh, 2017), Any organic matter that remains undegraded during this process can be transformed into digestate, which is free of undesirable or toxic substances. This substrate can be further processed into compost through aerobic maturation, facilitating the production of edible fungi and serving as an organic amendment to mitigate the risks of agrochemical pollution. Additionally, it helps improve soil structure and fertility in agriculture (Ambaye et al., 2021). To ensure smooth functioning and optimize digestion performance, various parameters must be carefully controlled during anaerobic digestion, these include temperature (*T*), pH, volatile fatty acids (VFAs), volatile solids (VS), chemical oxygen demand (COD), nitrogen total Kjeldahl (NTK), total organic carbon (TOC), and organic loading capacity (OLC) (Beniche et al., 2021). This study reveals that slaughterhouse waste contains higher levels of organic carbon, leading to increased methane yield. Additionally, innovative combined heat and power engine technology plays a crucial role in maximizing the utilization of biogas for electricity and heating, thereby significantly reducing our reliance on fossil fuels.

Overview of biogas production by anaerobic digestion

Anaerobic digestion technology

Biomass represents a significant potential source of putrescible and fermentable organic matter. It is widely generated worldwide and can be a source of disturbance in nature (Weiland, 2010). Various types of biomass, including food waste, market waste, green waste, slaughterhouse waste, sewage sludge, animal manure, and more, can be used as substrates for biogas production (Braun, 2007). As a result, many studies have characterized organic waste, each with its unique compositions and properties, influenced by factors such as location, origin, cooking practices, and socioeconomic conditions (Iacovidou et al., 2012). Given the heterogeneous nature of this waste, complete pre-treatment is necessary, often achieved through mixing or shredding to break down the organic matter (Prabhu et al., 2016). This mechanical pre-treatment of organic waste is used to expedite the hydrolysis reaction, decrease the retention time during anaerobic digestion, and enhance the yield of biogas production (Eskicioglu et al., 2007).

The process of anaerobic digestion is a natural process where organic matter decomposes without oxygen, driven by numerous microorganisms (Beniche et al., 2021). It involves four metabolic stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (see Figure 1) (Veeken et al., 2000). Generally, this anaerobic digestion process is influenced by various physicochemical parameters (such as pH, conductivity, dry matter, and temperature) and nutritional parameters (like phosphorus, total nitrogen, and total organic carbon), and is affected by microbiological factors to manage microbial agents (Wang et al., 2014). In this context, it is important to assess the composition of organic components, including the total solids (TS) and VS content, total Kjeldahl nitrogen (TKN), total soluble phosphorus (TP), total soluble organic carbon (TOC), carbohydrates, hemicellulose, cellulose,

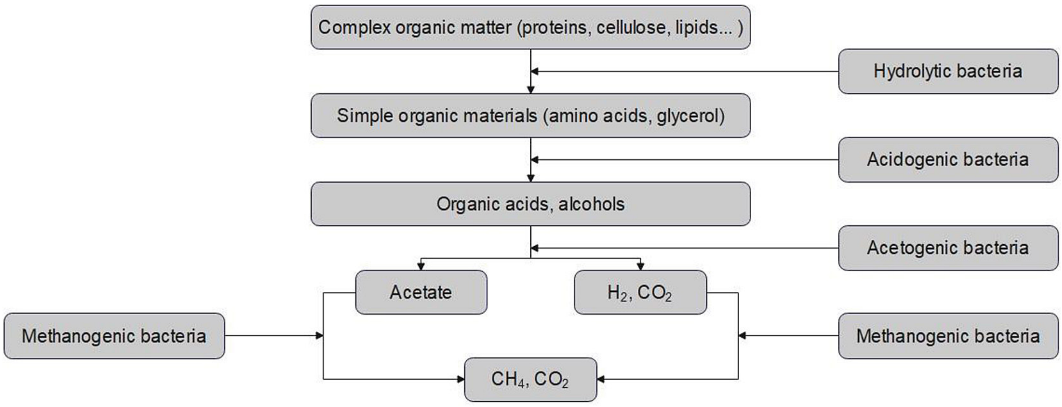

Figure 1. Stages of decomposition of organic matter (Morales-Polo et al., 2018)

lipid, protein, and lignin. To ensure the stability of this process and maintain a balanced carbon/nitrogen ratio, it is beneficial to add easily biodegradable co-substrates. This addition can increase biogas production with a higher methane yield (Braun, 2007). Table 1 provides an overview of the characteristics and composition of various organic matter.

Anaerobic digesters stage

At the forefront of anaerobic digestion's benefits is its ability to produce biogas abundant in methane (CH_4). This methane serves as a valuable resource for generating heat and electricity via internal combustion engines or micro turbines within a cogeneration facility (Sitorus et al., 2013). Consequently, many researchers have turned their attention to anaerobic co-digestion, combining two or more mixtures of substrates and co-substrates to stabilize the process, promote synergistic effects between microorganisms, and reduce greenhouse gas emissions and treatment costs (Siddique et al., 2018). Most often, anaerobic digestion systems use different types of digesters for biogas production (Weiland, 2010). The choice of an anaerobic digester type depends on the amount of substrate to be treated and valorized, the nature of the substrate, the fermentation conditions, the climatic conditions, and the investment capital (Tou et al., 2001).

Various new reactors have been designed and implemented in recent years (Ji et al., 2017), including batch reactors (Garcia-Peña et al., 2011), anaerobic sequencing batch reactors (ASBRs) (Hassib Bouallagui et al., 2009), continuous stirred tank reactor (CSTR) single phase (Shen et al., 2013), up-flow anaerobic sludge blanket (UASB) (Wu et al., 2016), hybrid anaerobic solid-liquid (HASL)(Arun Khardenavis et al., 2013), sequential batch anaerobic composting (SEBAC) (Fdéz.-Güelfo et al., 2010), semi-continuous anaerobic plug flow reactor (PFR) (Veluchamy et al., 2019), and a solid-state stratified bed (SSB) reactor (Chanakya et al., 2007). All of these have been used to treat organic waste. Each of these reactors has different methods for maintaining microorganisms, as well as generating methane and compost (Ji et al., 2017).

Several scientific studies have explored the digestion and co-digestion of organic waste, each with its unique focus. For instance, Di Maria et al. (2014) investigated biogas production from fruit and vegetable waste alongside mixed sludge as a co-substrate in a 100 – liter gas-tight anaerobic reactor equipped with a removable lid. They managed to reduce the hydraulic retention time from 14 days to approximately 10 days. Specifically, they observed that specific bio-methane production increased from around 90 NL/kg VS to a peak of about 430 NL/kg VS when the organic loading rate (OLR) was elevated from 1.46kg VS/m^3 day to 2.1 kg VSS/m^3 day. In a similar vein, Wang et al. (2014) examined the co-digestion of fruit and vegetable waste (FVW) with kitchen waste (KW) in continuously stirred tank reactors (CSTRs) at varying proportions. Their laboratory-scale experiments indicated that a 5:8 ratio of FVW to KW yielded higher methane productivity (0.725 L CH_4/g VS) with a hydraulic retention time (HRT) of 10 days. Another study by Ros et al. (2013) focused on co-digestion, this time involving sludge along with freshly chopped artichoke waste. They conducted their experiments in a continuously stirred anaerobic reactor operating at 8–10 rpm with a working volume of 300 liters over 55–71 days. Their findings demonstrated an increase in biogas production, averaging 354 ± 68 L·kg^{-1} of dry matter per day, with methane content exceeding 70%.

Table 1. Composition of various organics wastes (Shen et al., 2013; Raynal et al., 1998; Verrier, 1987)

Variable	pH	VS (g·kg^{-1} FM)	TS (g·kg^{-1} FM)	TKN (g·kg^{-1} FM)	Carbohydrates (g·100 g^{-1} VS)	Hemicellulose (g·100 g^{-1} VS)	Cellulose (g·100 g^{-1} VS)	Lipid (g·100 g^{-1} VS)	Protein (g·100 g^{-1} VS)	Lignin (g·100 g^{-1} VS)
Tomato	4.56	56.2	62.5	1.74	74.19	3.81	12.43	3.81	19.30	2.70
Pepper	5.06	106.6	113.9	2.37	77.77	2.50	8.07	4.41	13.91	3.91
Persimmon	5.94	198.6	204.4	1.11	96.66	2.45	3.19	0.18	2.80	0.35
Peach	3.76	125.9	132.9	0.89	93.18	2.66	4.71	0.73	5.52	0.66
Lettuce	5.6	30.4	31.3	–	–	–	–	–	-	–
Pawpaw	5.5	114.4	116.5	–	–	–	–	–	-	–
Pineapple	3.5	99.2	102	–	–	–	–	–	-	–
Banana	5.0	176.4	181.2	–	–	–	–	–	-	–
Orange	3.8	153.2	149.4	–	–	–	–	–	-	–
Potato	–	105.5	119.2	–	–	–	12.9	–	-	–
Carrot	–	82.9	90.4	2.0	–		16.1	–	-	–

MATERIALS AND METHODS

Collection and characterization of the substrates and inoculum

Poultry droppings and bleed water were collected from the Had Soualem farm in Casablanca, while green waste co-substrates were obtained from the Faculty of Science garden at Ain Chock. All waste materials were collected in February 2023. Generally, these co-substrates were added to the anaerobic digestion process to enhance biogas production by increasing the fermentable organic matter content. Additionally, to stimulate microbial activity in the digester, we introduced an inoculum derived from sewage sludge from the wastewater treatment plant in Mediouna, Casablanca. The characteristics of these substrates, co-substrate, and inoculum were detailed in Table 2.

Chemical analysis

The following parameters were analyzed in the different materials used as substrates, co-substrates and inoculum: T (°C), pH, conductivity (µS/cm), dissolved oxygen (mg/L), nitrate NO_{3^-} (mg/l), nitrites NO_{2^-} (mg/l), chloride Cl^- (mg/L), total chemical oxygen demand COD_t (mgO_2/L), total soluble organic carbon TOC_t (mg/l). These analyses were conducted following the APHA (American Public Health Association) Standard Methods.

Experimental design

A new study based on the production of biogas from Had Soualem poultry droppings was carried out in a CSTR infinite mixture digester with a capacity of 1.5 liters, effectively closed with a residence time of between 20 and 30 days. The quantity of poultry droppings substrate introduced into the reactor is 0.024 kg/d (0.015 kg of turkey per day and 0.009 kg of chicken per day). The agricultural grass waste content of co-substrate is 0.0006 kg/d diluted with slaughterhouse bleeding water at a rate of 0.3 m^3/d, It is recommended that these conditions be regularly monitored, adjusted if necessary and appropriate management practices implemented to maintain efficient operation of the digester.

For initiation of the anaerobic digestion process on a scale laboratory, the digester was inoculated with a quantity of GAL solution that contains a mixture of 25 g of glucose, 12 g of sodium acetate, and 11 ml of lactic acid in 500 ml of distilled water. During the process, we added various fillers to the digester, ranging from 0.25 g/L to 2, 5 g/L. This activation phase lasted around 15 days. After this phase came the adaptation of the substrate from the poultry droppings. Generally, the reactor was fed with a mixture of 1 g/L of GAL solution and substrate. Then we increased the loading by 1 g VS/L using a mixture comprising both the synthetic GAL solution and the substrate after we increased gradually the percentage of substrate until we reached 100% of the substrate. This phase lasted 20 days. During this period, we monitored the sample characteristics and biogas production for each load of GAL solution added, until we were using only pure substrate. The volume of this biogas was measured using the volume of water ejected from the tank.

Biogas compositional analysis

In an experimental digester, daily quantitative monitoring of biogas production has been established to track its progress. This digester was connected to a cylindrical gasometer with a capacity of one liter, constructed in the OSEV laboratory. The quantitative monitoring system involves connecting the gasometer to a graduated beaker of the same volume. Overall, the quantity of water released into the beaker represents the quantity of biogas produced (Fig. 2). Additionally, we assessed the composition of the biogas using a portable biogas detector of the X-am 5000 model. As part of our study, we monitored the biogas production with each load introduced into the

Table 2. Characteristics of substrates, co-substrates, and inoculum in Had Soualem (Morocco) Region

Specification	T (°C)	pH	Conductivity (µS/cm)	Dissolved oxygen (mg/L)	NO_{3^-} (mg/l)	NO_{2^-} (mg/L)	PO_4^{3-} (mg/L)	Cl^- (mg/L)	TOC_t (mg/L)	COD_t (mgO_2/l)	C/N
Poultry droppings	23.2	8.8	1962.2	–	120.23	20.252	22.56	135.7	7.645	20630.6	22.2
Green	22.5	7.26	1940	–	1.256	5.854	–	90.4	32.25	13206.5	20.1
WWTP sludge	23.5	7.35	2735	4.97	15.05	40.90	1.556	417.1	521.3	29562.98	14.5
Slaughterhouse bleeding water	–	6.83	3549	2.65	3.35	0.15	2.34	165.5	50	19437.4	20.7

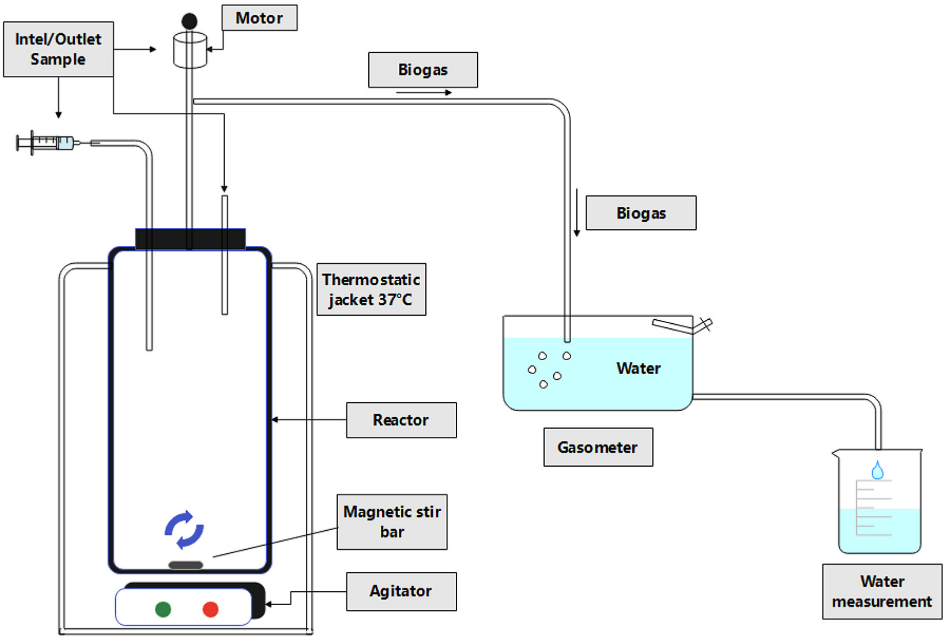

Figure 2. Schematic of CSTR digester used in laboratory OSEV

digester. Throughout the production process, we accurately calculated the amount of methane produced using the one-order nonlinear regression kinetic model simulation. This continuous monitoring enabled us to optimize our operating parameters and adjust our practices to maximize biogas production with high methane yield (Kocak, 2022).

Kinetics study

Kinetic modeling plays a pivotal role in comprehending the optimal technology for methane production within AD. They have provided key information for digester design, methane production prediction, and reactor performance optimization (Zhen et al., 2015). To analyze the findings from the anaerobic digestion experiments, we employed a one-order nonlinear regression kinetic model. This model established an analytical connection between the methane volume produced and the digestion duration and was derived and employed to assess the extent of process inhibition (Ikram Beniche et al., 2019). It is represented as follows (Borja et al., 1993):

$$G(t) = G_0 \times (1 - e^{(-kt)}) \qquad (1)$$

where: $G(t)$ – cumulative methane yield at digestion time t (ml), G_0 – methane potential of the substrate (ml), K – specific rate constant for methane production (day^{-1}), t – digestion time (days).

$$K = K_G \times X \qquad (2)$$

where: K_G – specific kinetic constant of methane production (L/g. day), X – concentration of biomass (g/L).

In parallel, we employed an alternative model Gompertz to estimate methane production rates in a continuously stirred tank reactor (CSTR) anaerobic digester. Typically, the modified Gompertz model is employed to establish the correlation between cumulative methane production and the minimum time required for methane production. In this context, Ji et al. (2016), examined the kinetics of anaerobic digestion of various plant wastes in the Yunnan region of China using logistic and Gompertz models, as well as other models. Both models exhibited good fit with experimental data, but a significant difference was noted in the maximum methane production rate values. Among these models, the modified Gompertz equation showed better consistency with experimental data compared to the logistic model. Moreover, Gnaoui et al. (2020), investigated two kinetic models, namely the logistic model and the modified Gompertz model. The findings indicated that both models demonstrated good fitting capabilities, with the modified Gompertz model being deemed more suitable than the logistic model. Assuming that the rate of biogas production under mesophilic conditions corresponds to the growth rate of methanogenic bacteria in the digester. The

Gompertz model equation is calculated as follows (Fernández-Rodríguez et al., 2023):

$$G(t) = P.exp(-exp\left(\frac{R_{max}}{P}(\lambda - 1) + 1\right)) \quad (3)$$

where: $G(t)$ – cumulative methane produced (mL/g) at a time t (day), P – maximum biogas yield potential (mL/g), R_{max} – maximum methane production rate (mL/g/d), λ – latency phase (day), *exp* – mathematical constant (2.71828).

DISCUSSIONS AND RESULTS

Stability of the process

In Had Soualem, the stability of the process was observed at the end of each load by varying the pH and the biodegradability in the reactor substrates. As shown in the first graph in Figure 3, the evolution of the pH value during the AD process, the pH value remains stable throughout the digestion period with values ranging between 7.1 and 7.6. Indicating that there is no need to add a solution to the digester to balance the process (Deepanraj et al., 2015). In addition, this stability could confirm that the CSTR digester process is working well (Nzila, 2017). Furthermore, assessing the biodegradability of co-substrates was an essential operational variable for stabilizing the process. As shown in the second graph in Figure 2, the biodegradability value increases until it reaches the maximum yield of 54% for the 1.5 g VS/L load and then declines as there is less readily available biodegradable organic matter in the substrates. This variation indicates that the microbial activity in the process is higher. In all cases, pH and biodegradability vary with the load added to the digester. However, the values found for these parameters are in agreement with those obtained by Li et al. (2013).

Biochemical methane potential assays

Figure 4 summarizes the methane production results obtained during the experiment for the mixture of selected substrates and co-substrates, including the methane rate per day. Poultry droppings and grass waste have a high methanogenic potential up to 75%. Thus, during anaerobic digestion, the production of biogas increases significantly during the HRT cycle indicating a lower retention time in higher biogas production. As well as the waste mixture also contains an optimum C/N ratio which favors the increase of methane yield during anaerobic digestion.

The experiment was conducted at the OSEV laboratory of the FSAC and lasted for 30 days. The collected data were used to plot a graph showing the evolution of the volume of biogas and the cumulative volume of methane, which occurred in three phases. The initial phase showed low methane production and gradual evolution, reaching up to 1,200 m^3 biogas units, with 66.3% methane, due to a pH decrease during the hydrolysis and acidogenesis phases. The subsequent phase saw faster biogas and methane production due to pH neutralization. In the final phase, methane production slowed until stabilization,

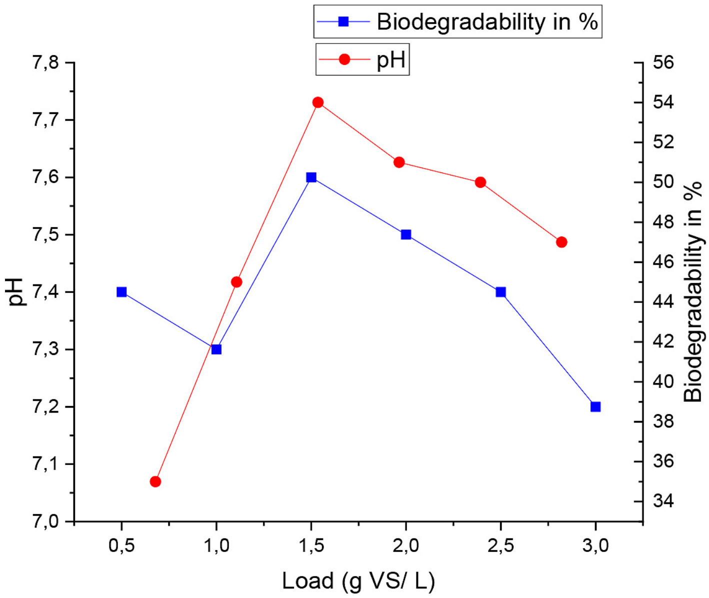

Figure 3. Variation of pH and the biodegradability with load

followed by a partial decrease. The findings suggest that the biodegradable substrate became less available for bacteria, indicating reduced interactions between organic matter and microorganisms (Bouallagui et al., 2009).

Several scientific works dealt with the co-digestion of organic waste and each study had its main objectives, Dornelas (2017), studied the effect of thermal treatment of poultry waste on biogas production based in two experiments, one without pretreatment where the quantified volumes ranged from 8.9 to 41.1 L of biogas and the second with pretreatment from 6.7 to 33.9 L. It was concluded that the reuse of poultry waste led to an increase in biogas production (Dornelas et al., 2017). Liu et al. (2009), studied the production of biogas from food and green wastes and their mixing using anaerobic batch digesters at mesophilic (35 ± 2 °C) and thermophilic (50 ± 2 °C) temperatures, the results found that the yields of biogas and methane from mesophilic digestion were lower than the yields obtained at the thermophilic temperature which were 430, 372, and 358 mL/g VS, and the methane yields were 245, 206 and 185 mL/g VS. In this context, Zhao et al. (2022), studied the effect of adding chicken manure (CM) on the anaerobic digestion systems of Enteromorpha and green waste (GW) as a co-substrate, to enhance bio-methane production. The maximum rate of bio-methane production showed a 49.9% improvement in the co-digestion of (CM:GW = 1:3). Figure 4 shows variation in biogas volume and methane content as a function of time in day in Had Soualem.

Kinetic parameters estimation

The aim of kinetic modeling is to investigate the kinetic parameters involved in methane production through the anaerobic digestion process. To characterize the kinetics of our experiment and enable comparison between various kinetic models used for methane production, we employed previous models to fit the experimental data. Figure 5 illustrates the evolution of both the accumulated methane volume and the methane kinetic volume using two modified models: first-order nonlinear regression and Gompertz, over time for the substrate under examination. This study is conducted on poultry droppings in Had Soualem using nonlinear regression and Gompertz. The observations reveal a variation in the experimental volume of methane between 70 and 75% CH_4. Nonlinear regression calculations indicate a theoretical range of 69.9913% to 74.9923%, while those obtained by the Gompertz model vary between 69.9954 and 74.9987%. The correlation coefficients for these models are between 99.855% and 99.934%, respectively. Based on these results, it can be concluded that the Gompertz model provides a better fit to the experimental data compared to the one-order nonlinear regression model. According to Budiyono et al. (2014), assessed the effectiveness of the modified Gompertz and first-order kinetic models in predicting biogas yields compared to the measured biogas yields. They found that the difference between the predicted and measured biogas yields was higher with the first-order kinetic

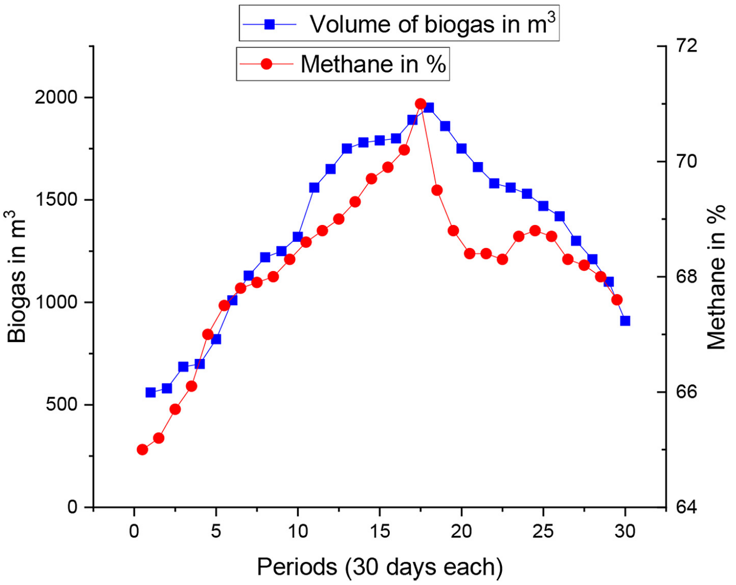

Figure 4. Variation in biogas volume and methane content as a function of time in day in Had Soualem

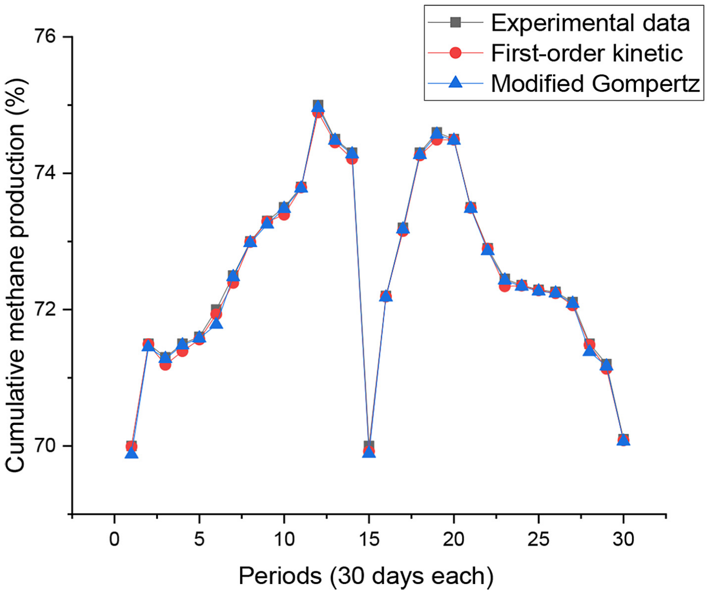

Figure 5. Temporal variation in experimental cumulative methane production and application of first-order nonlinear regression and Gompertz models

model ranging from 1.54% to 4.70% compared to the modified Gompertz model ranging from 0.76% to 3.14%. Consequently, it was concluded that the modified Gompertz model exhibited a better fit for substrates derived from vinasse.

Beniche et al. (2019), studied methane production through anaerobic digestion of dairy sludge from the industry. Their kinetic study found that the standard deviations of G_m and K_G remained below 5% and 15% respectively, indicating high precision in experimental measurements. Additionally, with constant R_2 values at 0.99, this strongly suggests that the proposed model aligns well with the collected data, confirming its reliability and accuracy in assessing energy recovery potential.

CONCLUSIONS

Anaerobic digestion under mesophilic conditions presents a promising avenue for harnessing energy from poultry waste in Had Soualem via methane generation. The research findings reveal that the poultry droppings exhibit a significant ratio of C/N, measured at 22.2%, enhancing their biodegradation and methane generation capabilities. The observed data demonstrates a consistent escalation in methane yield, peaking at 75% over the experimental period. The data indicates a steady increase in methane production over time, reaching up to 75%. This trend can be attributed to the degradation of organic matter, resulting in the generation of biogas within 30 days. To validate methane production kinetics, we conducted a thorough analysis utilizing modified Gompertz and first-order models. The modified Gompertz equation effectively aligns with empirical observations, offering valuable insights into methane production dynamics and facilitating optimization strategies for sustainable waste-to-energy initiatives in agricultural settings such as Had Soualem. Implementation of this technology not only mitigates waste volume but also harnesses organic substrates for the generation of renewable energy, including electricity, heat, or both, through cogeneration systems.

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