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Effect of organic loading on anaerobic digestion of cow dung: Methane production and kinetic study

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A B S T R A C T

Organic loading influences the effectiveness of producing biogas through anaerobic digestion. This study set out to investigate the effect of organic loading on the anaerobic mesophilic digestion of cow dung, the parameters involved in the digestion process and to evaluate the kinetics. Anaerobic digestion of cow dung at different organic loading (gVS/L) of 14 gVS/L, 18gVS/L, 22 gVS/L, 26 gVS/L and 30 gVS/L were investigated. Increasing the organic loading increased the methane yield of the cow dung. The highest cumulative methane yield was observed at 30 gVS/L with 63.42 mL CH_4/gVS while the highest biogas yield was reported at 192.53 mL/gVS with the highest methane content of 89%. In addition, the modified Gompertz model equation with an R^2 of 0.9980 demonstrated strong consistency and a good fit between predicted and experimental data. The high number of substrates added to the systems when increasing the organic loading increased the λ and slow down the nutrient transport and hydrolysis. This study provides current information on the effects of organic loading on the anaerobic digestion of cow dung in batch mode, including experimental conditions and operational parameters.

1. Introduction

The population of cows is estimated to be 1.5 billion worldwide and the global demand is predicted to increase to 74 million tons by 2023 [1,2]. Malaysia's recent increase in demand for dairy products is linked to increased livestock manure volume generation. Cows also produce the highest volume of manure in Malaysia with a yearly contribution of 5.45 million tons out of a million tons of livestock production [3]. Apart from the production of milk and meat, dairy cows are the primary producer of livestock manure, with a massive amount of cow dung, accounting for most of the greenhouse gas (GHG) emissions. In addition, the improper waste management techniques of cow dung have resulted in the escape of dangerous pathogens, GHG, and airborne ammonia, thereby leading to environmental issues [4,5]. Cow dung is toxic to the environment and causes nutrient imbalance and contamination because of its high

Abbreviations: GHG, greenhouse gas; VS, volatile solid; TS, total solid; TAN, total ammonia nitrogen; VFA, volatile fatty acids; TCD, thermal conductivity detector; GC, gas chromatography.

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nitrogen and phosphorus content, as well as traces of harmful elements.

Due to the fossil fuel depletion, a readily available, inexpensive, and environmentally friendly renewable energy source is necessary [6–8]. Manure produced about 55–65% of methane that responsible for the global temperature rise 21 times higher than carbon dioxide when released into the atmosphere [9]. Manure is a good feedstock for methane-rich biogas since it has the necessary methanogenic bacteria that are pertinent for anaerobic digestion and can be used as a substrate or inoculum [10,11]. Biogas with a high methane content is produced using a variety of feedstocks through anaerobic digestion, and up to 75% methane (CH_4) are produced through the process [12].

Several research on the utilisation of cow dung for renewable energy production have recently been conducted [13–16]. Abdeshahian et al. [3] reported that manure is a potentially inexpensive and sustainable source of energy in Malaysia for generating bioenergy and electricity. Specifically, the authors reported that manure can yield 4589.49 million m^3/year of biogas, which can generate an energy of approximately 8.27×10^9 kWh/year. Additionally, a techno-economic evaluation of a cow dung-based anaerobic biofilm reactor used in an on-farm biogas system dung was assessed [17]. Meanwhile, the biogas obtained from the anaerobic digestion of cows contains a high methane percentage [3], corresponding to a biogas production of 934.54 mL/gVS with a potential of 22.56 kWh of electricity generated. Similarly, high methane content is achieved by using cow dung as the main substrate in a fixed dome digester [18]. While in semi-continuous anaerobic digester, cow dung were reported to produced 77.32 L/kg VS of biogas with methane percentage of 57.23% [19]. Comparisons of biogas produced from different manures previously revealed that cow dung produced 204 mL/gVS of gas with more than half of the volatile solid (VS) removed [20]. According to the authors, average methane yield of cow dung (204 mL/gVS) is higher than goat (159 mL/gVS) and horse dung (155 mL/gVS). Nevertheless, co-digesting of cow and horse manure for biogas production has been studied and proven to improve biogas production from 5.1 L/g VS using only horse dung to 13.8 L/g VS when co-digesting horse and cow dung [15]. The anaerobic digestion of cow dung at various organic loading achieved the highest biogas yield of 270 mL/gVS, which is slightly higher than the result obtained by Kafle and Chen [20,21]. These studies suggest that cow dung is a promising substrate for biogas production due to the prevalence of beneficial bacteria that can promote anaerobic digestion.

The amount of VS to be fed into the digester is indicated by organic loading. It is crucial to the viability of the microorganisms and their maximum activity. An inappropriate organic load could fail accelerated hydrolysis and acidogenesis compared to methanogenic activity [22]. According to the German standard (VD1 4630), the concentration of substrate should be around 10 gVS/L [23,24]. Raposo et al. [24] posited that the concentration should be maintained at a range of 12–20 gVS/L since the process might be overloaded if the concentration is too high. The total loading in the substrate and inoculum should be between 20 and 60 gVS/L, with a total solid (TS) content of not more than 10% [25]. Total ammonia nitrogen (TAN), free ammonia, and volatile fatty acids (VFA) accumulate due to the effect of excessive organic loads may inhibit the methanogenesis [21,26]. The biodegradability of the substrate and methane production decreased when the organic load was increased to 64 gVS/L. Meanwhile, the organic loading depends on the feedstock characteristics. Lower organic loading may elicit nutrient deficiency in the microbes, whereas higher organic loading may result to the VFA accumulations in the system, which may subsequently inhibit methanogenesis and even process failure [27,28].

Thus, this study aims to elucidate the effects of organic loading on the anaerobic digestion of cow dung in batch modes, such as experimental conditions and operational parameters. This study was designed to fill the research gap on (1) the latest information required to operate batch anaerobic digesters, (2) provide anaerobic digestion process variables including pH, TAN, and VS affecting the whole process and (3) define the define biogas production of cow dung at different organic loadings and using Modified Gompertz kinetic model to elucidate the behaviour of the digestion process.

2. Materials and methods

2.1. Sample collection and preparation

Ladang 16 in Universiti Putra Malaysia provided the cow dung used as the studied substrate. Impurities were excluded by using 1 mm screens (Retsch, Germany) to screen the cow dung after it had been combined with equal portions of tap water. The substrate was then kept in an airtight container and plastic bottles, and chilled at 4 °C until use. The inoculum was collected from fresh manure that had been kept for over a month in an anaerobic environment. Before the anaerobic digestion process, the inoculums were stored in a mesophilic condition inside a water to allow the bacteria to acclimatise. The inoculums were kept in the same condition as the substrates.

2.2. Anaerobic digestion configuration and operational parameters

The 125 mL bottles used for each batch digestion test had an aluminium cap and a rubber stopper to secure them during the experiment. The bottles were maintained at 37 ± 1 °C in a water bath (Memmert, Germany). Cow dung was digested anaerobically at various organic loadings based on VS (14, 18, 22, 26 and 30 gVS/L). A 25 mL substrate was filled with the inoculum to yield a working volume of up to 100 mL. Thereafter, the substrate's pH was reduced to neutral using 2 M sodium hydroxide (HmbG, Malaysia) or 2 M hydrochloric acid (37%, JT Baker). Subsequently, the serum bottles were sealed after being subjected to 2 min of nitrogen gas flushing at a 3 L per minute flow rate. The batch testing was performed in triplicates for each batch test for a period of 40 days. The gas production was measured daily, and before collecting the gas, the bottles were manually mixed daily and submerged for the substrate level to be below the water level. Fig. 1 demonstrates the water displacement method used to calculate the volume of the gas produced. To remove the remaining traces of carbon dioxide in the gas, a carbon dioxide scrubbing unit was used, with 3 M sodium hydroxide as the scrubber. The biogas produced from the inoculum was subtracted from the gas produced in the serum bottles and the produced biogas yields were expressed in terms of the gas produced divided by the amount of VS added [29].

2.3. Analytical methods

The substrates were characterised before the anaerobic digestion procedure. For the TS, VS, and TAN, the standard procedure for water and wastewater examination was used [30]. The TAN test was performed by centrifuging the substrates for 15 min at 5000 rpm in a centrifuge (Kubota, Japan). The obtained liquid part was then collected to execute the analysis according to the standard procedure #4500-D [21]. The CHN628 Series Carbon, Hydrogen, Nitrogen Determinator (LECO, USA) was employed to analyse the carbon and nitrogen, and the results were used to calculate the C/N ratio of the substrate. Meanwhile, a pH metre (pH5SS Spear pH Tester, IONIX, Singapore) was used to determine the pH value. The composition of the biogas was identified using gas chromatography (GC), which was integrated with a thermal conductivity detector (TCD) analyser (Agilent 6890 N, United States) fitted with a fused silica capillary column (30 m × 0.53 mm). The gas sampling was collected as soon as passing through the carbon dioxide remover, and the GC sampling was conducted every 10 days. Methane yield was calculated using the GC data based on the method described by Hamzah et al. [31].

2.4. Kinetic study

The experimental data was kinetically examined using the Modified Gompertz model equation. The kinetic study presents various crucial data for the anaerobic digestion process, including the maximum biogas yield. Kinetic models have been created that allow for the prediction of the actual anaerobic digestion process to particular operating conditions [32]. The modified Gompertz equation [1] was used in this study to explain the behaviour and process of anaerobic digestion of cow dung [21]. The assumption of this model is that the biogas production rate is corresponding to the methanogenetic microbial particular rate of growth in the anaerobic digestor makes the equation suitable for the production of biogas in a batch mode [33].

$$B = B_0 \exp\left\{-\exp\left[\mu.\frac{e}{B_0(\lambda - t)} + 1\right]\right\} \frac{}{()} \tag{1}$$

where B is biogas production yield (mL/gVS), B_0 is the maximum biogas yield (mL/gVS, μ is the maximum biogas production rate (mL/gVS.day), e is exp (1), λ is the lag phase period and *e* is an Euler's function with a value of 2.71828. Microsoft Excel's solver tool was used to calculate the kinetics constants B, B_0 and λ.

3. Results and discussion

3.1. Substrates properties

Table 1 depicts the substrates and inoculum characteristics, which were evaluated before starting the process. Anaerobic digestion studies require accurate substrate characterisation since it affects the biogas production and the process stability. Cow dung yielded 35.03 g/L of VS on a dry basis. TS was observed to be 44.73 g/L, indicating that these substrates contain significant moisture as evident by the low TS measurement. Additionally, the C/N ratio of 17.25 indicated that cow dung contains high nitrogen with low carbon content. The C/N of cow dung was reported between 16 and 25 [28]. Cow dung's C/N ratio is suitable for anaerobic digestion, as the literature suggests a C/N ratio of 15–30 for anaerobic digestion [34,35]. The pH of cow dung is 6.9. A previous study also reported that the ideal pH range for improved microbial development was from 6.8 to 7.2 [28]. Since methanogenic bacteria prefer a neutral environment, the substrate pH was modified to 7 prior to digestion [22]. On the other hand, the TAN content of cow dung is 925.40 mg/L. The inoculum contained a VS of 19.27 g/L, a pH of 7.47, and a TS of 25.91 g/L.

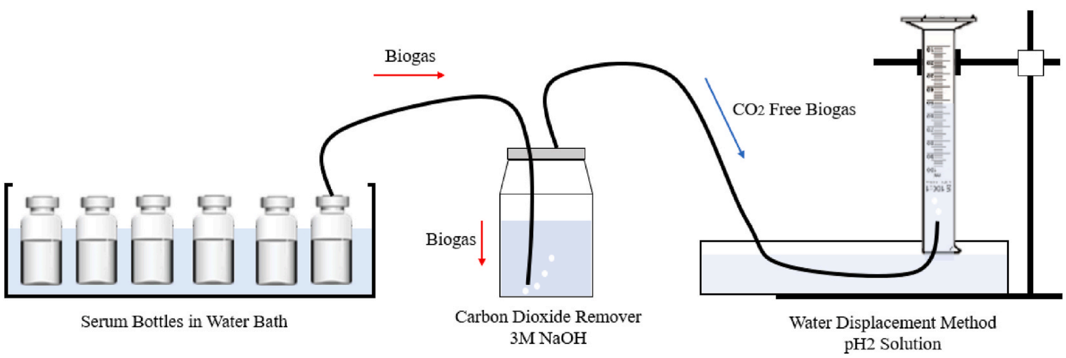

Fig. 1. Schematic diagram of Anaerobic gas collecting setup.

Table 1
Characteristics of the substrate and inoculum.

Parameters	Cow Dung	Inoculum
Total Solid (g/L)	44.73 ± 0.17	25.91 ± 0.75
Volatile Solid (g/L)	35.03 ± 0.21	19.27 ± 0.49
TAN (g/L)	0.93 ± 1.98	NA
Moisture Content (%)	95.52 ± 0.02	97.41 ± 0.08
Ash (%)	22.05 ± 0.69	25.62 ± 0.62
Carbon (%)	39.95 ± 0.04	NA
Nitrogen (%)	2.32 ± 0.02	NA
pH	6.90 ± 0.03	7.47 ± 0.03
C/N	17.25	NA

Notes: NA-Not Available.

3.2. Biogas and methane production

Fig. 2 depicts the cumulative biogas yield from mono digestion of cow dung at different organic loadings (gVS/L). Similar daily trends were observed at 14, 18 and 22 gVS/L. When the organic loading was increased to 22 gVS/L, biogas production began quickly and produced a significant amount of biogas. A higher organic loading level than 26 gVS/L slowed the early digestion process of the biogas production. The 26 and 30 gVS/L require more time to create a substantial volume of gas as compared to 14 gVS/L to 22 gVS/L. Lower organic loading (14–22 gVS/L) results in a significant increase in daily biogas generation in less than 13 days. Similar results were noted by Li et al. [21] in which a longer lag phase was observed at high organic loading due to an increase in the organic matter within the reactor. Moreover, the degradation process is longer in microbes due to the higher availability of organic matter. The shorter lag phase is favourable in assessing the efficacy of the anaerobic conversion process [36].

Previous studies also demonstrated that the shorter lag phase indicates that the amount of time required for active methanogenesis to start has been significantly reduced [11,15]. The presence of water is responsible for the decrease in biogas production that occurs alongside an increase in organic loading. Water promotes hydrolysis and reduces the restriction of mass transfer of the particulate substrate, allowing bacteria to grow, move and transport nutrients [37,38]. Thus, at higher organic loadings, low water content slows the transport process. Slower microbial activities reflect slower biogas production [37].

As shown in Table 2, the highest biogas volume for the mono digestion of cow dung was 433.19 mL (30 gVS/L), while the lowest was 222.68 mL (14 gVS/L). Despite the early stages (First 15 days) of biogas generation being slow, the methane (CH_4) yield for 30 gVS/L was the highest (61–89% of CH_4). The lowest methane content was observed at 14 gVS/L, with only 24–85% CH_4. In this study, high methane content (61–89% of CH_4) was reported in cow dung as compared to the literature. Previous studies found that mono digestion of cow dung produced 50–60% of methane at 32 gVS/L [36]. Anaerobic digestion of cow dung can also be improved by co-digestion with other substrates. Anaerobic digestion of cow dung with pineapple waste at a ratio of 2:1 improved the methane content up to 83–94% [31]. Likewise, anaerobic co-digestion of cow dung with other livestock wastes such as horse dung has the potential of producing the high biogas production [15]. The present study suggests that co-digesting cow dung and other wastes should

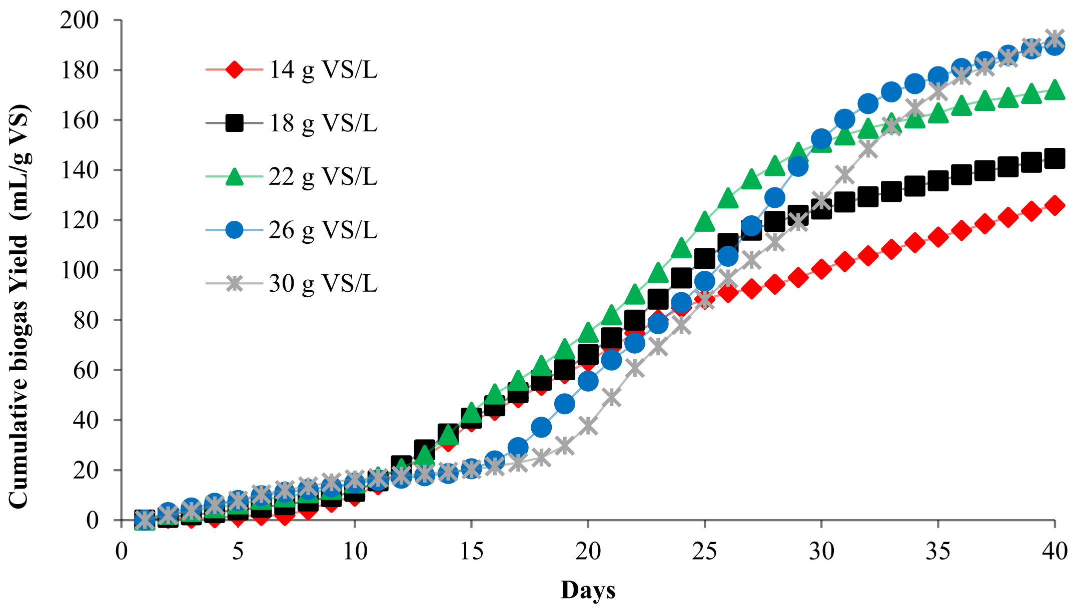

Fig. 2. Cumulative biogas production of the anaerobic digestion process at different organic loading.

Table 2
The summary of the biogas production of anaerobic mono digestion under various organic loadings.

Organic Loading (gVS/L)	Biogas Volume (mL)	Biogas Yield (mL/gVS)	Methane Content (%)	Methane Yield (mL CH_4/gVS)
14	222.68	125.81	24–85	31.37
18	273.33	144.62	35–88	38.30
22	346.00	172.14	43–88	50.43
26	404.30	189.81	56–87	60.71
30	433.19	192.53	61–89	63.42

be a better approach to producing high biogas value. The other strategy to improve methane content in biogas is to incorporate other purification units such as hydrogen sulfide remover, a biogas with lower carbon dioxide and hydrogen sulfide content is preferred to enable injecting the biogas into the public gas network grid [16,31].

Fig. 3 and Table 2 present the highest cumulative methane yield with 63.42 mL CH_4/gVS at 30 gVS/L. Methane yield increases as per increasing the organic loading of the substrate within the reactor. In contrast, different patterns were reported by Li et al. [21], whereby methane production was reduced when its organic loading increased from 8 gVS/L to 64 gVS/L of the mono digestion of manure due to increasing levels of TAN in the digester. The difference in the results might be due to the TAN value. Increasing manure loading inside the digester increases the TAN concentration, which at a certain level may heighten the formation of methanogenesis inhibitors. Specifically, inhibition of TAN occurs at a concentration higher than 1700–2000 mg/L [39,40].

3.3. Impact of digestion on process parameters

Several anaerobic mono-digestion process parameters, including the starting and final slurry, were assessed. Table 3 depicts the pH, VS, and TAN values. Resultantly, increasing the organic loading led to an increase in the final slurry pH. The pH levels ranged from 8.50 to 8.55. Considering that the ideal pH level for methanogenic bacteria activity is between 5.5 and 8.5, the pH level observed in the present investigation remained within the acceptable range [41]. The pH inside the reactor will drop during the early stages (hydrolysis and acidogenesis), but will then rise due to the TAN concentration at the end [42,43]. At high pH, the potential of TAN inhibition increases due to the accumulation of unionized ammonia, which subsequently increases its toxicity. The microbial activity of methanogenic bacteria is also inhibited as the value approaches the inhibitory level [26].

The highest TAN concentration (Table 3) in current study was 947.33 mg/L, which is much less than the 1700 mg/L inhibitory limit [39]. Thus, no TAN inhibition occurred in this study since the TAN did not exceed the inhibition level. Hence, selecting an appropriate organic loading for the reactor is crucial to maintain process stability. Several issues such as operational failure, accumulation of VFA and inhibition of TAN have been associated with improper organic loading inside the anaerobic digester [21,26]. TAN values can be reduced by co-digestion of cow dung with pineapple wastes. For instance, the TAN of the co-digestion process was reduced to 597.33 mg/L from 941.73 mg/L upon using only cow dung as the main substrate [31]. The 26 gVS/L loading revealed the maximum VS removal from the mono digesting procedure with 28.35% removal, followed by 28.28% for the loading of 18 gVS/L. VS removal in anaerobic digestion is often used as a biodegradability indicator [44]. High organic matter removal is indicated by high VS removal. Lower VS removal was observed in this study due to insufficient carbon content and low VS removal is often observed in mono

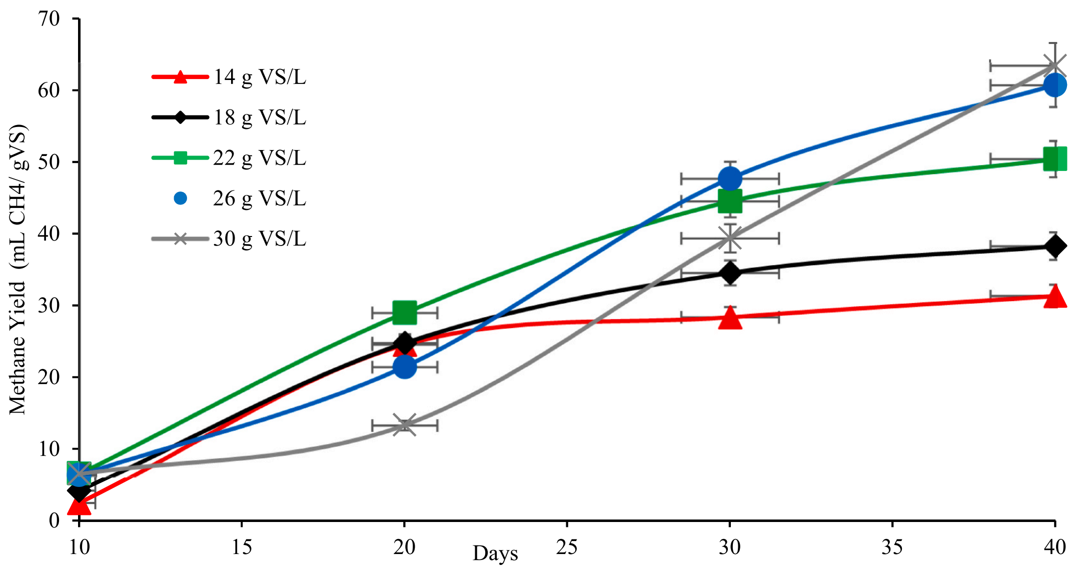

Fig. 3. The methane production from mono digestion at different organic loading.

Table 3
Slurry parameters for anaerobic mono digestion with various organic loadings.

Ratio	VS Removal (%)	pH		TAN	
gVS/L		Initial	Final	Initial (mg/L)	Final (mg/L)
14	25.67 ± 0.34	7	8.52 ± 0.03	452.67 ± 7.05	501.20 ± 5.60
18	28.28 ± 1.91	7	8.50 ± 0.07	547.87 ± 8.55	622.53 ± 8.55
22	26.82 ± 2.64	7	8.55 ± 0.05	719.60 ± 5.60	824.23 ± 11.66
26	28.35 ± 0.72	7	8.54 ± 0.02	735.47 ± 13.23	896.00 ± 32.29
30	10.87 ± 4.82	7	8.55 ± 0.06	795.20 ± 19.60	947.33 ± 11.66

digestion [45]. It was reported that co-digestion of manure with carbohydrate-rich substrates improve the substrate's degradability and increase VS removal [22,28,31]. The study by Li et al. [21] reported higher VS removal of cow dung ranging from 33.2% to 38.1%. Higher VS removal is desired, and it is the indicator of the high amount of organic matter converted to biogas.

3.4. Kinetic study

Using the modified Gompertz equation, the experimental cumulative biogas yields, predicted methane yields, lag phases, maximum biogas production rate (μ), and maximum biogas production yields (B_0) were calculated from the results of the anaerobic digestion of cow dung at various organic loadings. Table 4 provides a summary of the findings. The B_0 cow dung was at 30 gVS/L. The μ varied from 5.61 to 8.46 mL/gVS.day. The model had correlation coefficients (R^2) ranging from 0.9930 to 0.9980. Therefore, the performance of the cow dung showed good viability and consistency between the actual findings and kinetic model estimation. The fact that R^2 values were so near to one further demonstrated that the kinetic model was strongly associated with the actual production of biogas. Other than that, the production of biogas and optimization using response surface methodology also gave a higher prediction of the model with an R^2 value of 0.9998 [10]. Previously, Li et al. [21] reported the highest R^2 of 0.9974 from the cone model that fits well with their data. The Gompertz model, which is frequently employed in the simulation of biogas accumulation, has a reputation for being a good empirical non-linear regression model [17]. This model presupposed that the rate at which methane is produced in a batch digester corresponds to the particular rate at which methanogenic bacteria proliferate [20].

The difference between predicted and experimental results for 14, 18 and 22 gVS/L are 2.31, 7.99 and 10.41%, respectively. The deviation for 26 and 30 gVS/L are 19.41 and 33.57%, respectively. Previously, the kinetic model of dairy manure and other livestock waste showed the highest deviation at 70.88% observed in the first-order kinetic model [21]. Small deviation values are desired to signify that biogas yields are well simulated. High deviation from this study resulted from incomplete degradation that occurs at high organic loading due to the availability of active microorganisms that can still produce biogas [17]. As illustrated in Fig. 4, when organic loading went from 14 to 30 gVS/L, the lag phase (λ) lengthened from 9 to 15 days. As organic loading increases, the increase of λ is due to the number of substrates added to the systems. The high solid content in the systems reduced the water content, slower the bacterial growth and subsequently slow down nutrient transport and hydrolysis [46]. At greater loadings, the λ duration and hydrolysis rate of anaerobic manure digestion are longer [21]. This means that the bacteria needed a longer period to adapt and begin producing biogas that give big effect on the development and metabolic activities of methanogenic bacteria [33].

4. Conclusion

The amount of organic matter that is loaded into anaerobic digestion systems has a significant impact on the amount of methane that is produced from cow manure through anaerobic digestion. Both biogas and methane yields were at their highest levels at an organic loading of 30 gVS/L, and an increase in organic loading led to a rise in methane yield. Up to 89% of methane can be found in the biogas that is produced from cow dung. The effluents with the highest organic loading reflected the highest levels of TAN but not exceeding the levels that inhibit methanogenesis. The cumulative amount of biogas produced was analyzed using a modified version of Gompertz's kinetic equation, and it was fitted with both the experimental and predicted values. The observed longer period at high organic loading suggests that there is an increase in the number of degradable substrates. The present results might provide important information for future researchers regarding the quantity of material that should be loaded into the digester.

Author contribution statement

Adila Fazliyana Aili Hamzah: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Muhammad Hazwan Hamzah: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hasfalina Che Man; Nur Syakina Jamali; Shamsul Izhar Siajam: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Muhammad Heikal Ismail: Contributed reagents, materials, analysis tools or data.

Table 4
Kinetic parameters of modified Gompertz on anaerobic digestion of cow dung.

Organic Loading (gVS/L)	Modified Gompertz model				
	B (mL/g VS)	B_0 (mL/gVS)	μ (mL/gVS.day)	λ (day)	R^2
14	120.53	128.79	5.61	9	0.9975
18	145.55	157.18	6.74	9	0.9980
22	176.05	192.15	8.12	10	0.9969
26	198.20	235.52	9.14	14	0.9938
30	200.17	289.81	8.46	15	0.9930

Notes = B_0: maximum biogas production yield, μ: maximum biogas production rate, λ: lag growth phase time.

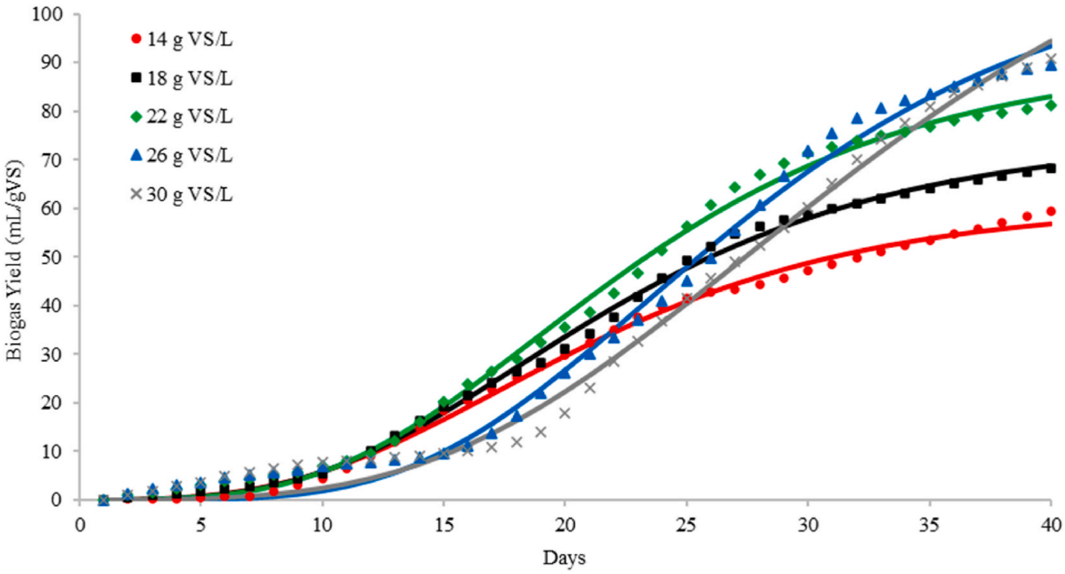

Fig. 4. Predicted biogas yield from the Modified Gompertz model.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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