

Article

Anaerobic Digestion of Horse Manure and Straw-Based Horse Litter[†]

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Abstract

The environmental sustainability of farms can be improved by the adoption of sound manure management techniques, with anaerobic digestion (AD) being one of the most promising: AD offers the production of renewable fuel, biogas or biomethane, and prevents the emissions of odors and greenhouse gases. While anaerobic digestion (AD) is widely implemented for treating dairy, pig, and poultry manure, limited interest has been directed toward horse farms. A laboratory-scale experiment was performed to assess the biochemical methane potential of horse manure and straw-based horse litter. The test was conducted using a BioReactor Simulator system (six digesters, 2.0 L each) at 38 °C for 40 days, using inoculum from a 1 MWe biogas plant fed with dairy cow manure and silages. Horse manure presented total solids (TS) of 19.4% and volatile solids (VS) of 90.7% TS, while straw-based horse litter showed a TS of 49.8% and VS of 90.0% TS. Methane yields of 48.2 NL/kg, 237.6 NL/kgTS, and 262.1 NL/kgVS were obtained for horse manure, and 82.1 NL/kg, 164.9 NL/kgTS, and 183.2 NL/kgVS for the straw-based horse litter. These results are of practical interest for the management of large horse facilities or for the co-digestion of these feedstocks in existing AD plants.

Keywords: anaerobic digestion; biogas; biomethane; energy; greenhouse gases; kinetic; horse manure; horse litter; methane; RNG

1. Introduction

Manure management represents one of the most important duties of a farm and can be considered of strategic importance in the pursuit of the environmental sustainability of farming. In fact, traditional farming effluent management techniques—such as open-air stockpiling or long-term storage on concrete floors—can determine undesired fermentations, especially with limited removal frequency or extended storage time, with the consequent emission of greenhouse gases and odors. Anaerobic digestion (AD) has been considered one of the most appealing technologies for the ecological transition and circular economy, for the benefits of preventing uncontrolled fermentations with reported prevention of the emissions of CO₂, N₂O and CH₄ of 77%, 78% and 90%, respectively [1], and of achieving significant reduction in odor impact of manure management [1–3] resulting in an improved acceptance of farming by the population. AD determines the production of biogas or biomethane, also called renewable natural gas (RNG), resulting from biogas upgrading typically by membranes or pressure swing adsorption (PSA): while biogas is



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mainly used to produce thermal and electric energy in combined heat and power units (CHP), RNG could be injected in the grid or used for transportation, even for heavy trucks after liquefaction, with a significant contribution to the ecologic transition. A secondary product of biogas upgrading to RNG is represented by CO₂, which is of potential interest for the food industry. AD has been implemented for years for the treatment of manures from dairy, cattle, pig and poultry farms, but limited interest was encountered by horse farms. Horse manure or litter are appreciated for their agricultural properties achieved after proper curing, and it is quite common to find them in packages on store shelves.

The most common management strategy is represented by land application [4], but in some cases composting is performed to achieve a further improvement of the quality of these products, especially thanks to the development of humic substances [5]. These solutions seem to be the most natural for solid manure and litter; however, unless a profitable local market is available, they entail significant operational costs (mainly for biomass transport and land application logistics) and infrastructural costs (related to compliant storage and composting facilities) [6].

An additional management option could be represented by thermal treatments such as gasification or combustion. Gasification could be appealing for the relatively small size of plants and of Syngas-powered CHP units (>20 kWe), but drying of the feedstock below 10% of moisture is a strict requirement and the process cannot be considered reliable on these feedstocks, which are heterogeneous by nature [2]. Direct combustion of litter based on wood chips presents less challenges and seems very promising, but pure manure and straw-based litter pose a risk for the combustion unit without pre-processing, due to high concentrations of S, K, Cl, and N [7]. Furthermore, limited Organic Rankine Cycle (ORC) applications are available under 250 kW of electric power, and combustion, in general, is often a cause of concern in the population with possible jeopardization of the success of the initiative [2].

The experiences of AD of equine effluents are quite limited, but in most cases the results seem promising [8–10]. Aspects that could negatively affect the diffusion of AD in horse farms are related to relatively small numbers of equines per facility typical of several areas of the world, even if tests were successfully performed in a family-size biogas plant in India [11]. In the case of extensive farming, furthermore, challenges are posed by limitations in efficiently collecting manure from paddocks [2].

Where horses are stabled in stalls, litter composed of straw, straw pellets, or most commonly wood chips is used; on one hand, litter should be capable of effectively absorbing urine, but on the other hand, it should not contain excessive dust that could affect the environment in the housing, which has a negative effect on animals' health. Horse litter should be changed on a daily basis, in order to limit the emission of ammonia in the stable, which has a negative impact on the respiratory health of the animals, and to keep a relatively dry environment for the hoofs. Typically, the removal is performed manually, but in some cases, scrapers are installed underneath the floor, extracting litter from the housing to form a pile outside the barn. Litter with a high content of lignin is not only hardly degradable in the AD process, but some authors report an inhibitory effect of AD by soft wood (pine chip) in equine litter, experienced in semi-continuous digesters and not replicated in batch, but still an object of concern [10]. Straw is more degradable than wood chips, which makes it the ideal candidate for litter destined for the AD process: straw-based litter proved to be very efficient in the AD process, as reported by Hadin and Eriksson [8].

A laboratory-scale experiment was conducted with the main objective of assessing the biochemical methane potential (BMP) of both fresh horse manure, and straw-based horse litter from a horse farm equipped with a scraping removal system. The tests were performed employing the BioReactor Simulator system (BPC Instruments, Lund, Sweden),

operating in batch and mesophilic conditions (38 °C). Additionally, a kinetic study was performed to evaluate the degradation rates of these substrates.

2. Materials and Methods

The effluents tested in the AD process were produced in a horse farm located in the province of Vicenza, Northern Italy. The farm breeds cutting horses, and provides boarding for several customers, with horses living in paddocks and others in stalls.

The barn (Figure 1) features two orders of 20 stalls with a central corridor (Figure 2). A litter removal system by scraper is installed under the floor of the housing (Figure 3), with one pit for each stall to allow operators to dispose of the spent litter. Each pit is equipped with a cover for safety. The cleaning of stalls is performed on daily basis. The material used as litter is represented by shredded straw.



Figure 1. Panoramic views of the horse farm.



Figure 2. View of the two lines of stalls served by the manure scraper.



Figure 3. Views of a horse stall, of the scraper pit and of the extraction/discharge of the litter.

Horse manure used for the experiment was represented by fresh solid feces promptly collected from the floor after production, while horse litter was collected from the external discharge pile that receives the product from the scraper (Figure 3).

The sampling was performed at about 30–40 cm of depth in order to collect representative material by avoiding the surface portion that could be partially dried by air (Figure 4). The scraper system operates on a daily basis. Horse litter was manually size-reduced by shortening the fiber of residual hay.



Figure 4. Close-up of horse manure (left) and horse litter (right) used in the test.

The inoculum used to prepare the mixture for the tests was represented by digestate collected from the post-fermenter (i.e., second stage digester) of a 1 MWe biogas plant treating dairy cow manure and corn silage and operating in the upper range of mesophilic conditions, at about 40 °C.

The tested substrates, inoculum and produced digestates were analyzed to determine total solids (TS), volatile solids (VS), NH_4^+ , pH, and redox potential following the Standard Methods for Examination of Water and Wastewater [12], carbon (C) and organic nitrogen (N_{org}) determined by CHN Vario Micro Cube (Elementar GmbH, Langenselbold, Germany), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) by the Van Soest detergent system, total acidity (FOS) and total alkalinity (TAC) by the

Biogas Titration Manager (Hach-Lange, Düsseldorf, Germany), and acid composition by high LC-20AT precision liquid chromatograph (HPLC, Shimadzu Corporation, Kyoto, Japan). Chromatographic separation provided high sensitivity with a limit of quantification sufficient to register trace organic acid levels, which were converted directly from software integration readouts.

Horse manure presented a TS content of 19.4%, while horse litter was characterized by TS of 49.8%. The VS content that resulted was relevant for both feedstocks, with values of 17.6% and of 44.8% on wet basis for horse manure and litter, respectively, corresponding to 90.7 and 90.0% relative to TS (Table 1). The content of carbon (C), organic nitrogen (N_{org}) and the consequent C/ N_{org} ratio results were similar for both materials, with C of 41.0%TS and 41.7%TS, N_{org} of 1.15%TS and 1.18%TS, and C/ N_{org} of 35.8 and 35.3 for horse manure and horse litter, respectively. These characteristics are comparable to the literature [10,11].

Table 1. Characteristics of inoculum and substrates. Values represent the mean of triplicate analytical determinations.

Parameter	Inoculum	Horse Manure	Horse Litter
TS (%)	5.6	19.4	49.8
VS (%)	4.5	17.6	44.8
VS (%TS)	79.5	90.7	90.0
C (%)	n.d.	41.0 [a]	41.7 [a]
N_{org} (%)	1.22	1.15 [a]	1.18 [a]
C/ N_{org}	n.d.	35.8	35.3
pH	7.73	n.d.	n.d.

[a] Values determined on dry basis; n.d. = not determined.

The inoculum presented TS of 5.6% and VS of 4.5% (79.5%TS), as reported in Table 1. An inoculum-to-substrate ratio of 2 (on a VS basis) was selected in accordance with standard international guidelines (VDI 4630) to ensure adequate buffering capacity and prevent process instability during the initial degradation stages [2].

The BMP test was performed at the University of Udine by means of the BioReactor Simulator system (BRS) (BPC Instruments, Lund, Sweden), a bench-scale equipment featuring digesters capable of semi-continuous or continuous operation, virtually simulating a full-scale AD plant. The system, depicted in Figure 5, is composed of 6 glass bioreactors of 2.0 L volume each, equipped with a mechanical mixing system operating at pre-set intervals, speed and direction (maximum 200 rpm). The experimental design utilized three single-run digesters without replicates, comprising two individual substrate treatments and one inoculum-only blank to subtract baseline methane production, as a preliminary screening step, described in this manuscript, prior to continuous operation. Specifically, the net methane production of each substrate treatment was calculated at each individual time point by subtracting the baseline methane generated by the inoculum-only blank, thereby ensuring that the final cumulative yields reflect solely the degradation of the organic matter in the horse manure and litter. While this single-run configuration serves as an effective preliminary screening, the absence of replicates limits the statistical confidence of the quantitative trends, and the results should be interpreted within this exploratory framework. During the 40-day test, the mixing system was operated for 20 s per min at a rotational speed of 40 rpm (20% of the maximum capacity).

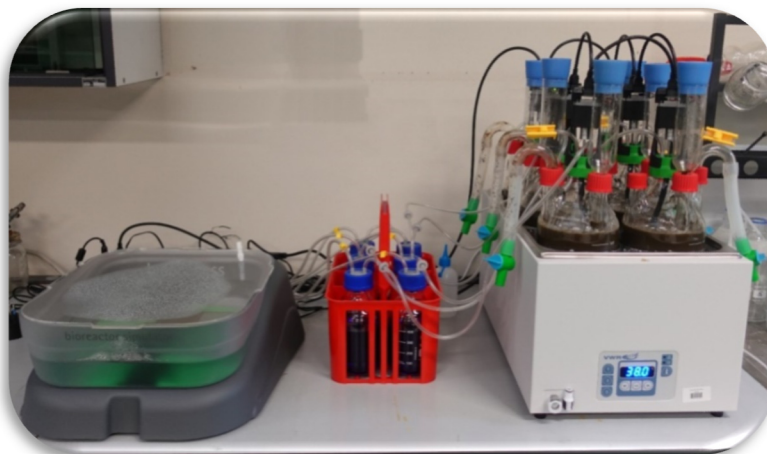


Figure 5. The BRS system used for the tests, with the gas measuring unit (left), the biogas purification units (center), and the digesters in the thermostatic bath (right).

The digesters were manually loaded with a mixture of inoculum and feedstocks and operated in batch mode. Temperature control is achieved by a thermostatic bath set at 38.0 °C. Each digester was connected to an independent gas line designed to convey the raw biogas directly into a dedicated chemical purification unit. This unit operated via a chemical scrubbing mechanism, wherein the biogas stream was brought into contact with a strongly alkaline solution. The alkaline interface efficiently trapped and removed the acidic gaseous fractions—principally carbon dioxide and hydrogen sulfide—via neutralization reactions, leaving a purified methane stream to be directed to the volumetric counter, consisting in flow-cell precision units (resolution 10 mL).

Kinetic Models.

The first-order kinetic model (Equation (1)) and the modified Gompertz model (Equation (2)) were tested to fit the experimental data of the cumulative methane yield (NmL CH₄/g VS).

$$Y(t) = Y_0(1 - e^{-kt}) \quad (1)$$

$$Y(t) = Y_0 \exp \left\{ -\exp \left[\frac{R_{\max} e}{Y_0} (\lambda - t) + 1 \right] \right\} \quad (2)$$

where

$Y(t)$ = estimated cumulative methane yield at time t (mL CH₄/g VS);

Y_0 = calculated maximum methane potential of the substrate (mL CH₄/g VS);

k = hydrolysis rate constant (1/day);

t = time (day);

$R_{\max}$ = maximum methane production rate (mL CH₄/g VS day);

λ = lag phase duration (day).

The first-order kinetic model is generally more accurate when hydrolysis represents the phase controlling the whole process (rate-limiting step) [13]; the modified Gompertz model, instead, is considered the best empirical non-linear regression model for describing the microbial cells' exponential growth rates, and in particular the lag phase duration [14]. The simple linear regression method was used for comparing the two models against the actual experimental data, and the hydrolysis constant k and the lag phase duration λ of the two models were assessed.

3. Results and Discussion

3.1. Methane Yield

The experiment was performed for a total of 40 days. The evolution of the AD process resulted regular, as depicted in Figure 6. The production of methane was quite progressive, with a higher production rate in the first 10 days of the process. The maximum daily production was recorded on day 7 for both feedstocks, with higher values for horse manure. The methane yields were higher for horse litter relative to fresh input, with a value of 82.1 NL/kg compared to 48.2 NL/kg of manure, as a result of the higher content of solids in the litter. The yields relative to total and volatile solids, however, showed methane yields of 237.6 NL/kgTS, and 262.1 NL/kgVS for horse manure, and of 164.9 NL/kgTS, and 183.2 NL/kgVS for horse litter (Figure 7). This is related to the higher degradability of fresh horse manure, while horse litter contains fibers that reduce the general yield. Wartell and colleagues report similar results, with methane yields of 170 L/kg VS added for manure–straw litter, after six weeks, and 200 L/kg VS measured over 45 to 60 days for horse manure alone [10]. Kusch and colleagues [15] obtained 170 L CH₄ per kg VS added in six-week cycles with untreated material under optimized conditions. Methane production was increased after chopping the substrate, while pre-aeration resulted in decreased methane production. Böske and colleagues reported methane yields of 235.4 NL/kg VS from horse manure mixed with straw, and 78.1 NL/kgVS for horse manure with wood shavings [16], and Nitsche and colleagues report 222.3 and 233.0 NL/kgVS for horse manure and horse manure mixed with straw [7].

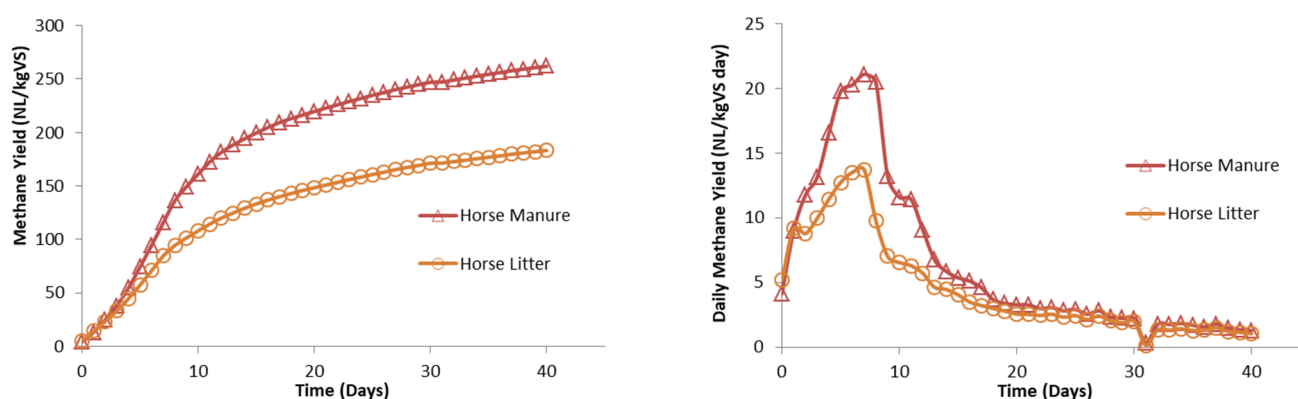


Figure 6. Cumulative and daily methane yields per kg of volatile solids.

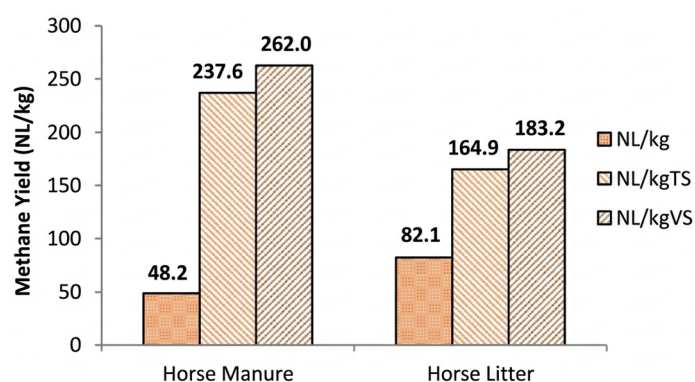


Figure 7. Final methane yields relative to wet mass, TS and VS.

3.2. Characteristics of Digestates

Digestate from horse manure presented a TS of 5.1%, VS of 3.9% (76.5%TS), while digestate from litter presented TS of 5.6%, VS of 4.2% (74.5%TS) (Table 2), values quite typical for a wet AD process [17,18].

Table 2. Characteristics of horse manure and straw-based digestates. Values represent the mean of triplicate analytical determinations.

Parameter	Digestate Horse Manure	Digestate Horse Litter
TS (%)	5.1	5.6
VS (%)	3.9	4.2
VS (%TS)	76.5	74.5
C (%TS)	37.9	37.5
N _{org} (%TS)	2.24	2.1
NH ₄ ⁺ (mg/L)	1920	2230
C/N _{org}	16.9	18.1
pH	7.55	7.63
NDF (%TS)	46.2	51.0
ADF (%TS)	34.2	39.9
ADL (%TS)	19.5	20.5
FOS (mg/L)	2600	2636
TAC (mg/L)	11,785	12,238
FOS/TAC	0.22	0.22
Redox (mV)	−370	−380
Lactic Acid (mg/L)	−	−
Acetic Acid (mg/L)	−	−
Propionic Acid (mg/L)	0.0045	0.0150
Isobutyric Acid (mg/L)	0.0190	−
Butyric Acid (mg/L)	−	−
Isovaleric Acid (mg/L)	−	−
Valeric Acid (mg/L)	−	−

An important parameter in AD is represented by the C/N ratio, with the optimal range being in the range between 20 and 30 to assure a correct balance between C and N availability: C is needed to produce methane, but values over 35 may indicate lack of nitrogen; on the other hand, values below 20 can indicate an excess of nitrogen, potentially leading to the development of ammonia. Horse manure and horse litter presented C/N ratios of 35.8 and 35.3, slightly above the upper limit, but this is not a real problem considering that the input represents only a fraction of the volume in a wet AD process [2,14,16]. It should be noted that the reported C/N ratio of approximately 35 was calculated based strictly on organic nitrogen, which accounts for the slight deviation from the standard optimal range of 20–30. To optimize this balance in full-scale operations, co-digestion with nitrogen-rich agricultural effluents or livestock slurries would represent an effective operational strategy. Furthermore, digestates from horse manure and horse litter presented C/N_{org} of 16.9 and 18.1, respectively, comparable to the C/N of 14.9 of the inoculum, indicating an effective digestion [2].

Digestate from horse manure presented pH of 7.55, FOS of 2600 mg/L, TAC of 11,785 mg/L, and FOS/TAC ratio of 0.22, indicating an effective degradation of volatile fatty acids by methanogenic microorganisms [2,17]. This result is also highlighted by the absence of lactic acid, acetic acid, butyric acid, isovaleric acid, and valeric acid, while propionic acid and isobutyric acid presented concentrations of only 0.0045 mg/L and 0.0190 mg/L, respectively.

Digestate from horse litter presented similar parameters, with a pH of 7.63, FOS of 2636 mg/L, TAC of 12,238 mg/L, and FOS/TAC ratio of 0.22, underlining an efficient anaerobic digestion process as well [2]. Also, in this case the detailed analysis of acids confirms the result, as highlighted by the absence of lactic acid, acetic acid, butyric acid, isovaleric acid, isobutyric acid and valeric acid, while propionic acid presented a concentration of 0.0150 mg/L.

3.3. Kinetic Modeling and Process Dynamics

The cumulative methane production curves and the corresponding kinetic fitting parameters are illustrated in Figure 8 and Table 3, respectively. Both the first-order and the modified Gompertz models demonstrated an outstanding capability to simulate the experimental anaerobic digestion process, yielding high coefficients of determination ($R^2 > 0.97$). Although the first-order model achieved a slightly higher overall coefficient of determination ($R^2 = 0.9927$ for horse manure and $R^2 = 0.9964$ for horse litter), the two mathematical approaches exhibited distinct phase-specific fitting behaviors. The modified Gompertz model proved to be more accurate in describing the initial stages of the process (days 1–5); its characteristic sigmoidal structure effectively accommodated the initial lag phase (λ), preventing the overestimation of early cumulative yields. Conversely, the first-order model tended to overestimate methane production during the first 3 days, but it achieved a better fit during the intermediate linear production phase (days 10–25) and accurately followed the late plateau phase (after day 25) for both substrates. The calculated hydrolysis rate constants (k) were 0.092 L/day for horse manure and slightly lower, 0.086 L/day, for horse litter. This minimal difference in hydrolysis velocity seems to confirm that, although horse litter contains significantly higher lignocellulosic fractions than pure manure, its degradation rate was not hindered. This phenomenon could be explained by the mechanical conditioning (horse hoof trampling in the stalls), which likely enhanced its accessibility to microbial enzymes. Furthermore, the maximum daily methane production rate ($R_{\max}$) reflected the higher availability of readily fermentable organic matter in the horse manure (21.1 NmL CH₄/g VS day) compared to the more structural horse litter (13.7 NmL CH₄/g VS day). The lag phase duration (λ) was very similar for both substrates, ranging between 1.63 and 1.65 days. This extremely short latency period indicates that the highly adapted microbial community of the inoculum faced no inhibition or toxicity upon integration to the equine effluents. The kinetic outputs achieved in this study are in robust agreement with the literature. For instance, Kun et al. [19] reported comparable first-order hydrolysis constants for livestock waste, citing 0.084 L/day for dairy manure and 0.068 L/day for horse manure. The slightly higher k -values obtained in our trials (0.092 and 0.086 L/day) underscore the high quality and optimal state of freshness of the collected effluents, as well as the high enzymatic activity of the inoculum used.

Table 3. Hydrolysis rate constant (k), lag phase duration (λ), and maximum methane production rate ($R_{\max}$) from the first-order kinetic model and the modified Gompertz model.

Substrate	First-Order		Modified Gompertz		
	k (1/day)	R^2	$R_{\max}$ (NmL CH ₄ /g VS day)	λ (day)	R^2
Horse Manure	0.092	0.9927	21.1	1.65	0.9849
Horse Litter	0.086	0.9964	13.7	1.63	0.9757

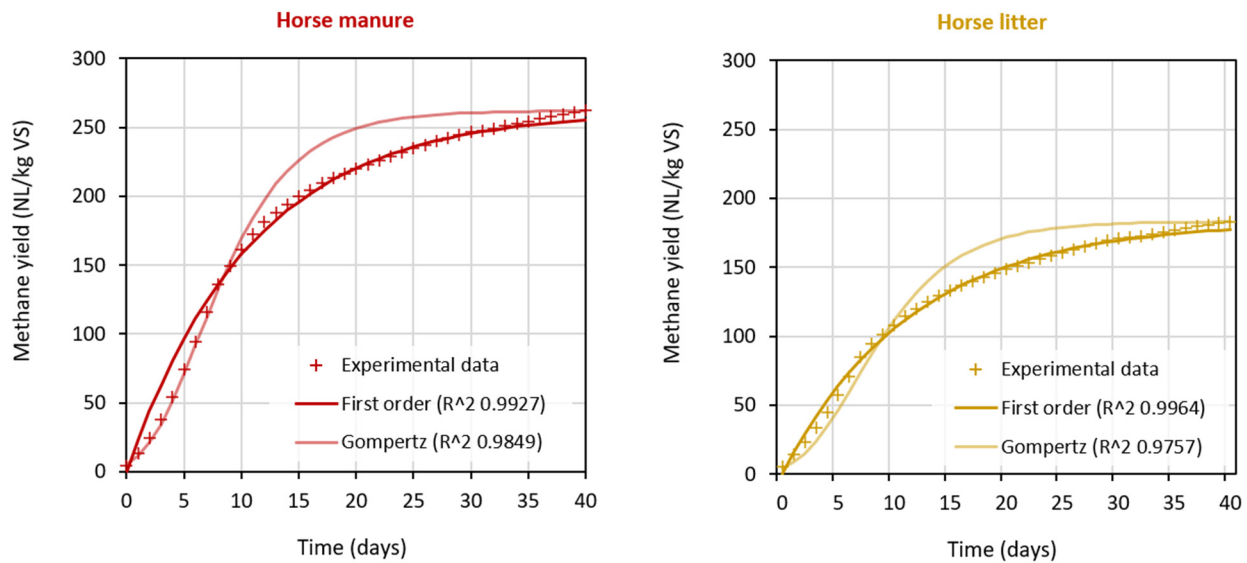


Figure 8. Cumulative methane yields: measured and models' predicted data.

4. Conclusions

This study assessed the biochemical methane potential (BMP) of fresh horse manure and straw-based litter under mesophilic conditions (38 °C) for 40 days using a laboratory-scale BioReactor Simulator system. The fresh manure (TS = 19.4%, VS = 90.7% TS) achieved methane yields of 48.2 NL/kg, 237.6 NL/kg TS, and 262.1 NL/kg VS. The horse litter (TS = 49.8%, VS = 90.0% TS) yielded 82.1 NL/kg, 164.9 NL/kg TS, and 183.2 NL/kg VS. Kinetic modeling showed that both approaches successfully simulated the process ($R^2 > 0.97$). While the modified Gompertz model precisely captured the initial lag phase, the first-order model provided an excellent overall fit, reflecting stable degradation kinetics across both matrices, likely favored by the mechanical conditioning in the stalls. Nonetheless, for practical plant operations, utilizing straw-rich horse litter will require extended hydraulic retention times (HRT) or the implementation of suitable biomass pre-treatments to overcome the structural limitations of lignin. These findings underline a significant energetic potential for both feedstocks, establishing baseline data that can support energy recovery in horse facilities or via optimized co-digestion in existing agricultural anaerobic digestion plants to eventually balance the carbon-to-nitrogen ratio and improve overall process kinetics. Furthermore, the frequent removal of litter and manure for anaerobic processing provides substantial environmental and operational benefits, including effective odor and fly control, thereby improving animal welfare and coexistence with nearby residential areas. While these batch screening results offer a reliable baseline, future work will focus on pilot-scale trials under continuous operating conditions and multi-sampling campaigns, which are essential to map operational parameters and process kinetics for full-scale plant engineering.

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