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Biochemical Methane Potential and Kinetic Parameters of Goat Manure at Various Inoculum to Substrate Ratios

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Abstract: Anaerobic digestion is a proven technology for managing manure while harvesting natural gas and digestate as a biofertilizer. The biochemical methane potential (BMP), biodegradability, and kinetic parameters of goat manure (GM) were investigated at different inoculum to substrate ratios (ISRs). The cumulative biomethane yields at the ISRs of 0.0, 0.3, 0.5, 0.8, 1.1, 1.3, and 2.6 were 191.7, 214.3, 214.9, 225.9, 222.1, 222.8, and 229.9 mL g_{VS}^{−1}, respectively. The biomethane yield at all ISRs was significantly higher than control (0 ISR). Above the ISR of 0.0, the biomethane yield was similar among all ISRs. The biodegradability of GM at the ISRs of 0.3, 0.5, 0.8, 1.1, 1.3, and 2.6 varied between 73.3% and 78.7% and was statistically similar. In total, 90% of the yield was observed in 31 and 32 days in control and all other ISRs, respectively. The modified Gompertz equation fitted very well ($R^2 = 0.99$) to the BMP of GM but predicted the lag phase (λ) of 3.2–5.2 days against observed 8–10 days among control and other ISRs.

Keywords: goat manure; inoculum to substrate ratio; biochemical methane potential assays; kinetic modeling



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1. Introduction

The high global warming potential, 25 times greater than CO₂, along with a lifetime of 12 years in the atmosphere, makes methane one of the largest contributors to the greenhouse effect [1]. The major atmospheric methane sources are anoxic, which include wetlands, paddy cultivation, ruminants, termites, biomass burning, leakages from energy generating systems, landfills, oceans, and hydrates, whereas the oxic source of atmospheric methane is terrestrial vegetation [2]. The enteric fermentation in domestic ruminants such as cattle, swine, sheep, and goats is one of the largest anthropogenic methane contributors. When both are combined, the agricultural sector is the largest atmospheric methane contributor to the atmosphere. Among the adverse environmental impacts of anthropogenic agriculture-driven activities is eutrophication, causing the formation of one of the world's largest dead zones in the Gulf of Mexico [2–6]. Methane and other air pollutants such as hydrogen sulfide, ammonia, and particulate matter are found to be at higher concentrations in the atmospheric air surrounding the animal manure storage tanks or lagoons during the summer months [7–10]. Among the animal manures, those with low moisture and high C:N values emit higher GHGs and more unpleasant odors [11], a characteristic of goat manure (GM) [12,13]. The United States is home to 2.58 million goats, while Asian and African countries host more than 90% of the world's goat population [14,15]. The United States Department of Agriculture (USDA) encourages the adoption of anaerobic degradation (AD) technology at animal farms [16]. Currently, there are 273 operational anaerobic digesters at dairy, cattle, and swine farms across the US [1]. Consequently, the methane emissions from manure tanks and lagoons have been harvested as bio-energy by employing this technology [1,7]. The process of AD is carried out by various bacterial

groups where organic matter is converted into biomethane or refined natural gas, which is a promising renewable energy source [17,18]. In addition to biomethane (carbon-neutral energy), the AD process produces less sludge, removes pathogens and odors more effectively, and yields digestate with high fertilizer value, thus having several advantages over aerobic digestion [19]. There are four major stages of the AD process, i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis [20–24]. Gujer and Zehnder [25] identified six distinct processes: hydrolysis of biopolymers, fermentation of amino acids and sugars, anaerobic oxidation of long-chain fatty acids and alcohols, anaerobic oxidation of intermediate products such as volatile acids, conversion of acetate to methane, and conversion of hydrogen to methane occurring in the anaerobic digesters. Several factors, such as inoculum, substrate, experimental and operational conditions, impact the microbial population carrying out these processes [26]. The animal manures are characterized by high nitrogen (inorganic) contents which cause process inhibition by releasing excessive ammonia in the digesters [27]. Another important operational factor impacting the process is the inoculum source and its ratio to the substrate or ISR [20,28–33]. Small-scale laboratory experiments or biochemical methane potential (BMP) assays provide useful information to better understand the effect of each factor.

There are no previous studies focusing on the effect of ISR on the BMP of GM. In this study, the BMP of GM was determined at different ISRs and the process efficacy was investigated by calculating biodegradability and kinetic parameters.

2. Materials and Methods

2.1. Characterization

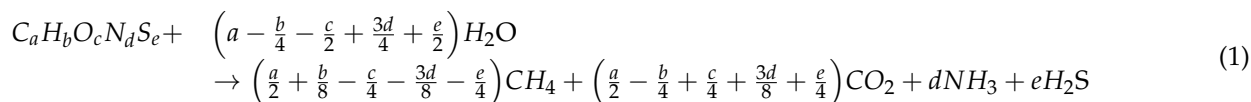
The International Goat Research Center and municipal wastewater treatment plant (WWTP, mesophilic), at Prairie View A&M University, served as the source of the GM and inoculum, respectively, for the BMP assays. After initial processing (dried at 80 °C, passed through Willy mill, and sieved (2 mm)), its proximate, ultimate, compositional, and chemical properties were analyzed in triplicates. For proximate analysis of moisture, total solids (TS) and volatile solids (VS) were determined by method 2540 [34] using a Lindberg Blue M electric furnace (Fisher Scientific, Pittsburgh, PA, USA). The elemental composition (C, H, N, and S) was determined with a Flash 2000 Organic CHNSO Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). For analysis, 2,5-Bis(5-tert-butyl-2-benzoxazolyl)thiophene with C, H, N, and S contents of 72.5%, 6.1%, 6.5%, and 7.5%, respectively, with utilized as a standard compound. The composition of cellulose + hemicellulose and lignin was determined by thermogravimetric analysis in a Perkin Elmer Diamond TG/DTG system. The HACH (Loveland, CO, USA) kits were used to determine total phosphorus (2,767,245), volatile acids (TNT 872), alkalinity (TNT 870), nitrate (TNT 836), and ammonium nitrogen (TNT 832). Pure compounds, 500 mg L⁻¹ PO₄³⁻, 1000 mg L⁻¹ CH₃COOH, 25,000 mg L⁻¹ CaCO₃, 500 mg L⁻¹ NO₃⁻, and 10 mg L⁻¹ NH₄-N, were diluted as needed for accuracy checks. The inoculum was de-gassed [35,36] for a week before BMP assays and proximate analysis [34].

2.2. Biomethane Potential Assays

The triplicate BMP assays were carried out in 250 mL serum bottles (Fisher Scientific, Pittsburgh, PA, USA). The organic loading rate (OLR) was 1% VS (1.98 g GM) for all treatments. The varied ISRs of 0.3, 0.5, 0.8, 1.1, 1.3, and 2.6 (inoculum volume, mL/g, GM total wt.) were obtained by adding 0.5, 1.0, 1.5, 2.0, 2.5, and 5 mL of inoculum, respectively. A total working volume of 100 mL in each treatment was achieved by adding deionized water (DI). Other procedures, including blanks, were the same as described in our previous studies [23,24,37]. More specifically, the blanks contained inoculum and all other ingredients except GM. The control bioreactors (0.0 ISR) contained GM and all ingredients except inoculum. The serum bottles were incubated at 36 ± 1 °C and the daily biomethane volume was recorded by the liquid displacement method [23], which absorbs the second major component of biogas, carbon dioxide.

2.3. Theoretical Maximum Methane Yield

The theoretical maximum biomethane yield (TMY) was determined from the chemical formula of GM ($C_{295.8}H_{603.0}O_{351.0}N_{12.6}S_{1.7}$) by modified Buswell Muller's [37] known as Boyle's equation [38] below (Equation (1)) as described in our previous study [23].



$$TMY = \frac{22.4 \times 1000 \times \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)}{12a + b + 16c + 14d + 32e} \quad (2)$$

where $a = 295.8$, $b = 603.0$, $c = 351.0$, $d = 12.6$, $e = 1.7$.

2.4. Process Efficacy, Simulation, and Data Analysis

The process efficacy was determined from the biodegradability (BD) of the experimental and theoretical BMPs, EMY and TMY, respectively. The process was also simulated by a modified Gompertz equation (Equation (3)), and data were analyzed as described in our previous studies [23,24,37].

$$P(t) = P_0 \times \exp \left\{ -\exp \left[\frac{R_m e}{P_0} (\lambda - t) + 1 \right] \right\} \quad (3)$$

$P(t)$ = The accumulated methane ($\text{mL g}_{\text{vs}}^{-1}$) at digestion time t (days)

P_0 = Maximum cumulative methane production ($\text{mL g}_{\text{vs}}^{-1}$)

R_m = Maximum daily rate of biomethane production ($\text{mL g}_{\text{vs}}^{-1} \text{ days}^{-1}$)

λ = lag phase (days), minimum time to produce biomethane

e = Mathematical constant 2.718

The model fit was evaluated by calculating the coefficient of determination (R^2) and the relative root mean square (rRMSE) as described by Kafle et al. [39].

The null hypothesis for the original research is that the inoculum concentration (volume) does not have any impact on the BMP of the GM and AD process (determined by fitting the experimental cumulative data to the modified Gompertz equation) and its efficacy. The null hypothesis ($H_0: \mu_1 = \mu_2 \dots$) for the original research was that the biomethane yields of control (μ_1 ; 0 ISR) and inoculum receiving bioreactors (μ_2 ; ISR treatments) in pairwise comparison are similar. The alternative hypothesis ($H_1: \mu_1 \neq \mu_2 \dots$) is that the yields are different from the control. The hypotheses were tested (three replications) with the general linear model (GLM) procedure of SAS (SAS[®] 9.2, SAS Institute Inc., Cary, NC, USA) with the Fisher's least significant difference or LSD test at 0.05 degrees of freedom. All data were analyzed in triplicate.

3. Results

3.1. Daily Biomethane Production

The highest biomass degradation rate or peak yields were observed between the 9th and 11th days in inoculum receiving bioreactors and control, respectively (Figure 1a). For a 0 ISR, a maximum biomethane of $23.6 \pm 0.8 \text{ mL g}_{\text{vs}}^{-1}$ was observed on the 11th day. The daily biomethane peaks of 31.7 ± 2.8 , 36.3 ± 7.9 , 30.8 ± 1.6 , 30.8 ± 8.3 , 36.8 ± 3.5 , and 29.5 ± 6.5 were observed for the ISRs of 0.3, 0.5, 0.8, 1.0, 1.3, and 2.6 on the 9th day, respectively.

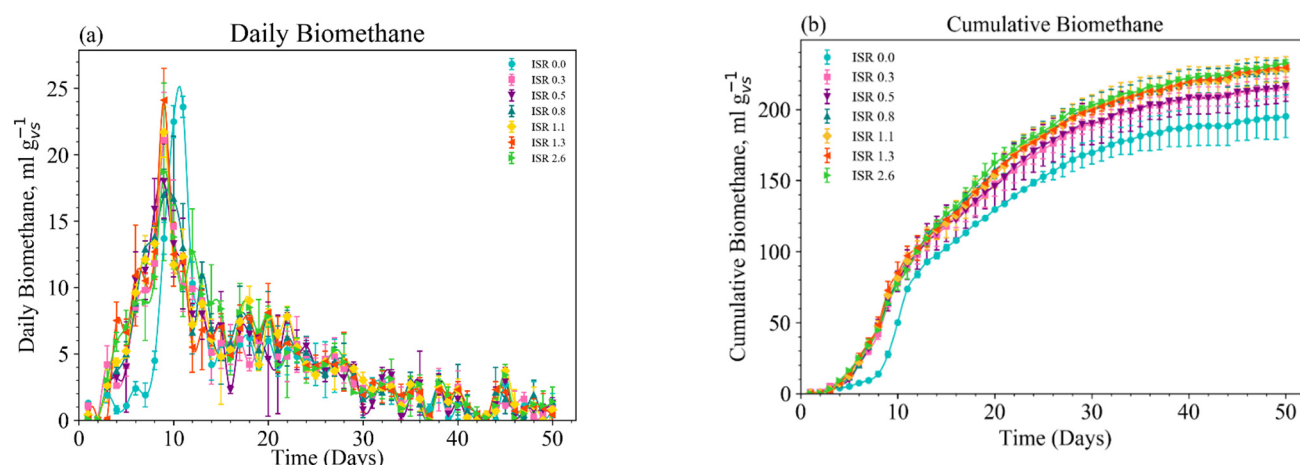


Figure 1. (a,b). Daily and cumulative biomethane yields (mL g_{vs}⁻¹).

The data points are means derived from triplicate values; the error bars represent standard deviations.

3.2. Cumulative Biomethane

From the cumulative biomethane chart (Figure 1b), it is evident that the BMP (50 days) increased with an increase in ISR. The BMPs of 191.7 ± 17.6 , 214.3 ± 7.8 , 213.1 ± 12.0 , 225.9 ± 8.9 , 222.1 ± 12.0 , 228.8 ± 2.2 , and 229.9 ± 2.9 mL g_{vs}⁻¹ were observed for ISRs of 0–2.6. The BMPs at all ISRs tested in this study were significantly higher ($p < 0.0001$) than the control (0 ISR). The BMPs at 0.3–2.6 ISRs were similar ($p < 0.0001$). The microbial population from digested sludge of a municipal WWTP was not acclimated to the substrates. The microbial acclimation to the GM during the hydrolysis stage might be the reason for an observed lag phase of about 4 days in 0.3–2.6 ISRs (Figure 1b). The technical digestion time (T_{80-90}) is the time to achieve 80–90% of the maximum yield. The T_{80-90} of 26–31 days was observed in control bioreactors, whereas it was 26–32 days for 0.3 ISR. The T_{80-90} for ISRs of 0.5 and 0.8 were the same (24–32 d), whereas it was 25–32 days for ISRs of 1.1–2.6. The addition of inoculum increased the cumulative biomethane to 11.7%, 12.0%, 17.8%, 15.7%, 16.2%, and 19.9% in 0.3, 0.5, 0.8, 1.1, 1.3, and 2.6 ISRs, respectively. As the treatment yields were significantly higher ($p < 0.0001$) than the control, the original null hypothesis that inoculum concentration does not affect the BMP outcomes was rejected.

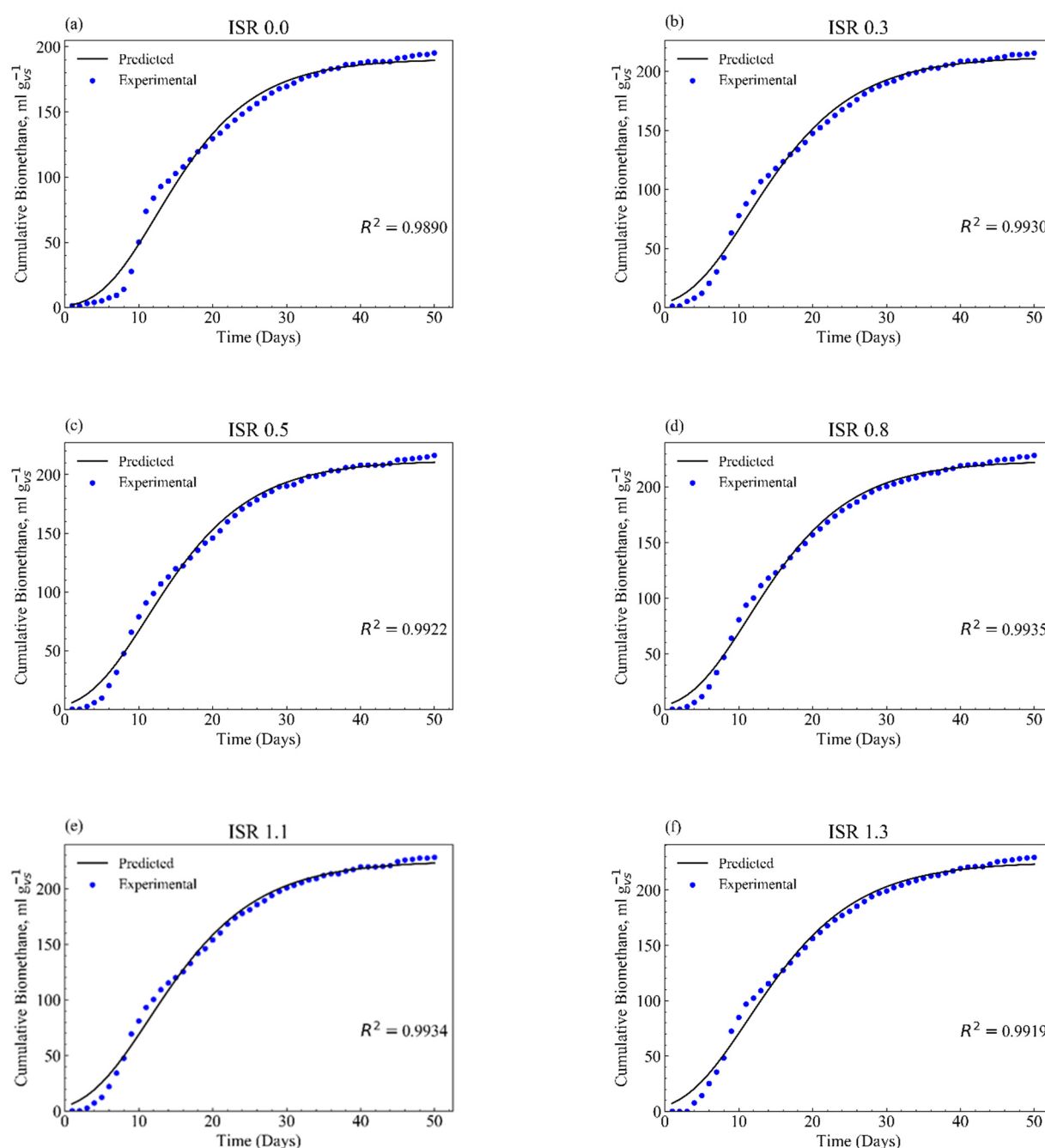
3.3. Methane Production Kinetics

As the simulated biomethane yields (P_0 ; Table 1) at all ISRs were significantly higher ($p < 0.0001$) than control, the alternative hypothesis was accepted. The model predicted that the maximum daily rate of biomethane production (R_m) was ($p < 0.0001$) similar in all ISRs, and varied between 9.8 and 10.9 mL g_{vs}⁻¹ days⁻¹, whereas, the experimental maximum daily biomethane production rates were higher and varied between 18.0 ± 2.8 and 24.1 ± 2.4 . The null hypothesis was accepted for the simulated R_m values. The addition of inoculum significantly lowered ($p < 0.0001$) the simulated lag phase (λ) from 3.4 to 3.7 (in 0.3 to 2.6 ISRs) over 5.2 ± 0.4 days observed in the control, leading to the acceptance of the alternative hypothesis. Of all the ISRs the model predicted, 3.2 and 3.7 days λ was closest to the observed 3 days in the ISRs of 1.3 and 2.6, respectively. The high coefficient of determination, R^2 , further supports that the equation fits well to the AD of GM at all ISRs for the simulated BMP (Figure 2). The R^2 value of control was significantly lower ($p < 0.0001$) than all other ISRs, leading to the acceptance of an alternative hypothesis. The R^2 was similar in the ISRs of 0.3, 0.5, 0.8, 1.1, 1.3, and 2.6 tested in this study. The lowest and highest rRMSE values of 0.536 and 9.083 were observed in the ISRs of 0.0 and 2.6, respectively.

Table 1. The experimental and simulated biomethane yields and kinetic parameters.

ISR	P_0 ¹	E ²	R_m ³	λ ⁴	R ²	rRMSE ⁵	BD (%)
0	186.6 ± 19.3 b	191.8 ± 17.6 b	9.8 ± 0.6 a	5.2 ± 0.4 a	0.988 ± 0.001 b	0.536	65.6 ± 1.6 b
0.3	211.0 ± 7.0 a	214.3 ± 7.8 a	9.9 ± 0.9 a	3.4 ± 0.2 bc	0.992 ± 0.002 a	1.250	73.3 ± 0.7 a
0.5	206.5 ± 8.2 a	214.9 ± 9.0 a	10.2 ± 1.2 a	3.7 ± 0.1 b	0.991 ± 0.002 a	3.644	73.7 ± 0.8 a
0.8	220.1 ± 9.4 a	225.9 ± 8.9 a	10.7 ± 0.5 a	3.6 ± 0.3 bc	0.992 ± 0.003 a	5.355	77.4 ± 0.8 a
1.1	218.6 ± 11.2 a	222.1 ± 12.0 a	10.0 ± 1.3 a	3.5 ± 0.1 bc	0.994 ± 0.002 a	2.222	77.4 ± 0.9 a
1.3	224.2 ± 3.5 a	222.8 ± 2.2 a	10.2 ± 0.2 a	3.2 ± 0.4 c	0.993 ± 0.000 a	9.083	78.3 ± 0.2 a
2.6	223.6 ± 4.9 a	229.9 ± 2.7 a	10.9 ± 0.8 a	3.7 ± 0.3 bc	0.994 ± 0.001 a	5.99	78.7 ± 0.2 a
LSD ⁶	18.7	18.7	1.6	0.7	0.002	-	5.9
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	<0.0001

The values represent mean ± SD of triplicates, and those sharing the same letter across a column are similar at $\alpha = 0.05$. ¹ Simulated biochemical methane potential (BMP) (mL g_{VS}⁻¹), ² Experimental BMP (mL g_{VS}⁻¹), ³ Maximum daily rate of biomethane production (mL g_{VS}⁻¹ days⁻¹), ⁴ Lag phase (d), ⁵ Relative root mean square error, ⁶ Least significant difference.

**Figure 2.** Cont.

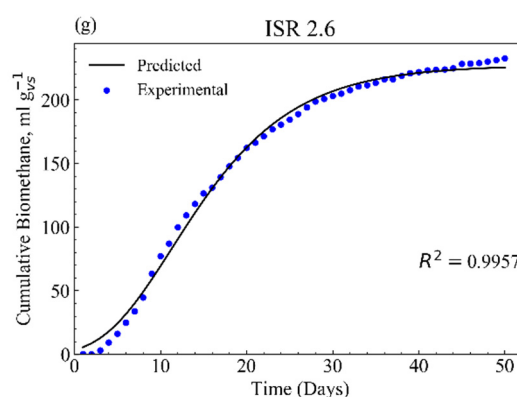


Figure 2. (a–g). The modified Gompertz model—simulated and cumulative experimental biomethane yields ($\text{mL g}_{\text{VS}}^{-1}$).

3.4. Biodegradability

Following the data in Table 1, $65.6\% \pm 1.6\%$ of the added VS was converted to biomethane (biodegradability) even without the inoculum. With the addition of inoculum, even at the lowest ISR of 0.3, the BD significantly increased ($p < 0.0001$) to $73.3\% \pm 0.3\%$. With the further increase in ISR, the BDs at 0.5, 0.8, 1.1, 1.3, and 2.6 ISRs were $73.7\% \pm 0.5\%$, $77.4\% \pm 0.8\%$, $77.4\% \pm 1.1\%$, $78.3\% \pm 1.3\%$ and $78.7\% \pm 2.6\%$, respectively, and stayed statistically similar ($p < 0.0001$). The null hypothesis was rejected ($p < 0.0001$), although the alternative hypothesis was accepted.

4. Discussion

While studying the effect of inoculum on the BMP of GM, we observed that the AD started even without the addition of inoculum (0 ISR), attaining a BD of $65.6\% \pm 1.6$. When passing through the goat digestive system, the AD of the manure may already have been taking place by the intestinal microbiome. The presence of goat intestinal microbiota might be the reason that the process started without the addition of inoculum in the BMP assays. The high concentration of $2795.0 \pm 63.6 \text{ mg L}^{-1}$ of volatile fatty acids (VFAs) in GM (Table 2) is an indication of the anaerobic microbial activity. The optimum pH and alkalinity values of 7.8 ± 0.3 and $5265.0 \pm 106.1 \text{ mg L}^{-1}$ of the GM might explain the smooth AD until completion of the BMP assays [20]. The substrate degradation in all ISRs followed a similar trend of steady decline after attaining the peak yields between 9 and 11 days (Figure 1a). Kafle and Chen [39] reported a similar trend and a daily peak value of 18 mL after 7 days AD of GM.

The BMP of GM varied between 214.3 ± 7.8 and $229.0 \pm 2.7 \text{ mL g}_{\text{VS}}^{-1}$ in the tested 0.3–2.6 ISRs in our study. The BMP in this study is similar to $221.9 \text{ mL g}_{\text{VS}}^{-1}$ reported by Zhang et al. [40] after AD of GM for 55 days at an ISR of 2.5 under a similar temperature of $35 \pm 1 ^\circ\text{C}$. On the volatile solid basis, the feed to inoculum ratios (F/I) tested in this study were 125, 66.7, 43.5, 33.3, 26.7, and 13.3 in ISR treatments of 0.3, 0.5, 0.8, 1.1, 1.3, and 2.6, respectively. An increase in the inoculum's volatile solid led to the increases of 11.7%, 12.0, 17.8, 15.7, 16.2, and 19.9%, in these treatments, respectively. In our previous study [23], we observed a BMP of $274.4 \pm 7.8 \text{ mg L}^{-1}$ at F/I of 28 for GM. Inoculum concentrations lower (125, 66.7, 43.5, 33.3 F/I) and higher (26.7 and 13.3) than the previous study were selected. We report the $223.6 \pm 4.9 \text{ mg L}^{-1}$ BMP of GM at the highest ISR of 2.6 (F/I of 13.3) in this study. Kafle and Chen [39] reported a BMP of $242.0 \text{ mL g}_{\text{VS}}^{-1}$ in 45 days at a F/I of 0.5 (on a volatile solid basis) for GM, which was lower than our previous study [23]. The inoculum is added as a source of diverse microbial populations (depending upon the sources), which multiply quickly under favorable conditions. These conditions vary with each experiment [20]. In our study, the increase in inoculum concentration increased the BMP, but it was not significant beyond the ISR of 0.3 (compared to 0.0 ISR). The BMP values of 147.4, 238.1, and $212.7 \text{ mL g}_{\text{VS}}^{-1}$ have been observed in dairy, swine, and poultry manures, respectively [12,13], having C:N ratios of 17.1, 10–12.5, and 8.3, respectively.

Although the optimum carbon to nitrogen ratio is reported to be 26–34 [13], it did not seem to affect the BMP in this study. The 80–90% total biomethane yields were achieved between 24 and 26 and 31 and 32 d, respectively, in all ISRs. However, the time to obtain 80–90% of the BMP has been reported as between 24 and 31 and 37 and 39 d, respectively, in previous studies [23,39], having *F/I* ratios of 28.0 and 0.5.

Table 2. Goat manure (GM) and inoculum characteristics.

Parameter	GM	Inoculum
Proximate analysis		
Moisture (%)	35.9	97.2 ± 0.3
VS (%)	52.8	1.5 ± 0
Ash (%)	11.3	1.5 ± 0.4
Ultimate analysis		
N (%-TS)	1.7	-
C (%-TS)	35.5	-
H (%-TS)	6.0	-
O (%-TS)	56.2	-
S (%-TS)	0.5	-
C/N	20.9	-
Compositional analysis		
Cellulose + Hemi-cellulose (%-TS)	72.4	-
Lignin (%-TS)	17.6	-
Elemental Formula	C _{295.8} H _{603.0} O _{351.0} N _{12.6} S _{1.7}	-
Chemical analysis		
pH	7.8 ± 0.3	
Alkalinity (CaCO ₃ , mg L ⁻¹)	5265.0 ± 106.1	
VFA (CH ₃ COOH, mg L ⁻¹)	2795.0 ± 63.3	
NO ₃ ⁻ -N (mg L ⁻¹)	9.0 ± 1.4	
PO ₄ ⁻ -(mg L ⁻¹)	992.0 ± 4.2	
NH ₄ ⁺ -N (mg L ⁻¹)	715.5 ± 154.9	

The maximum BD of 78.7 ± 0.2% observed in 2.6 ISR was similar to lower ISRs (except control or 0 ISR). In our previous studies [23,41], BDs of 33.5 and 94.5% were observed at an ISR of 2. The difference in values may be because of the difference in the time that manure was collected in addition to the different organic loading rates of 10.0% TS. The GM had a lignin content of 17.6% (Table 2). It is well documented that lignin, one of the most recalcitrant carbonaceous components, is not easily degraded during the AD process [39,42–44]. Wahid et al. [45] reported a BD of 29.4% in cow manure at an ISR of 1:1.

Of the various AD models [46], the process efficacy was determined by employing the modified Gompertz equations. The model-derived BMPs followed a similar trend to those of the experimental values. Among other kinetic parameters of GM, previous studies [23,37,45] reported a lag phase of 0–1.4 d, whereas in the current study, we report minimum and maximum values of 3.2 and 5.2 days in an ISR of 1.3 and control, respectively. We observed that the maximum daily rate of biomass degradation or R_m was statistically similar at all ISRs (including control), and it was between 9.8 and 10.9 mL g_{VS}⁻¹ days⁻¹. The R_m of 8.0 [41] and 10.6 mL g_{VS}⁻¹ days⁻¹ [23] for GM has been reported at an ISR of 2 in our previous studies.

The T₈₀ at all ISRs varying from 0 to 2.3 was 24 to 26 days, whereas 90% of the total BMP yield was attained in 7 to 8 additional days.

5. Conclusions

The results reveal that anaerobic digestion of goat manure starts without the addition of inoculum at optimum conditions, but its addition augments biomethane recovery. An increase in the ISR from 0.3 to 2.6 enhanced biomethane yield; however, the change was not significant. The modified Gompertz equation is a good fit for predicting BMP during the AD of GM at all ISRs. The T₈₀ at all ISRs and control varied between 24 and 26 d. The

T_{90} of 31 and 32 days was observed in control and all ISRs, respectively. The T_{80-90} (24–26 and 31–32 days, respectively) observed in this study can be used as HRT for continuous reactors. Our findings contribute significantly towards the understanding of anaerobic digestion of goat manure; it will thus help to design the digesters at goat farms and will help in achieving the higher goals of pollution control and renewable energy recovery via better manure management.

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