



# Optimizing anaerobic co-digestion of goat manure and cotton gin trash using biochemical methane potential (BMP) test and mathematical modeling

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## Abstract

Anaerobic co-digestion is widely adopted to enhance process efficacy by balancing the C/N ratio of the feedstock while converting organic wastes to biomethane. Goat manure (GM) and cotton gin trash (CGT) were anaerobically co-digested in triplicate batch bioreactors. The process was optimized and evaluated utilizing mathematical equations. The liquid fraction of the digestate was analyzed for nitrate and phosphate. The co-digestions with 10 and 20% CGT having the C/N ratios of 17.7 and 19.8 yielded the highest and statistically similar  $261.4 \pm 4.8$  and  $262.6 \pm 4.2$  mL/g<sub>VS</sub> biomethane, respectively. The biodegradability (BD) of GM and CGT was  $94.5 \pm 2.7$  and  $37.6 \pm 0.8\%$ , respectively. The BD decreased proportionally with an increase in CGT percentage. The co-digestion having 10% CGT yielded 80–90% of biomethane in 26–39 d. The modified Gompertz model-predicted and experimental biomethane values were similar. The highest synergistic effect index of  $15.6 \pm 4.7\%$  was observed in GM/CGT; 30:70 co-digestion. The concentration of nitrate and phosphate was lower in the liquid fraction of digestate than the feedstocks, indicating that these nutrients stay in the solid fraction. The results provide important insights in agro-waste management, further studies determining the effects of effluent application on plants need to be conducted.

**Keywords** Goat manure · Cotton gin trash · Biomethane potential assays · Kinetic modeling · Biofertilizers · Anaerobic digestion

## 1 Introduction

The systems where animals are raised under confined situations for more than 45 days a year are defined as animal feeding operations (AFO). Concentrated animal feeding operations (CAFO) is a term given by EPA to the large-sized AFOs and/or AFOs where animal wastewater (manure etc.) is stored in open ditches (manmade or natural) or released to the environment via water streams [1]. The animal manures stored in tanks or lagoons release large

amounts of methane and other air pollutants (hydrogen sulfide, ammonia, and other particulate matter) in the atmosphere during the summer months [2–5]. Consequently, CAFOs are regulated under the National Pollutant Discharge Elimination System of EPA [1]. Anaerobic digestion (AD) is widely adopted to convert organic matter into biomethane or refined natural gas (a form of bioenergy and potential alternative to fossil fuels) at such farms [6, 7]. As of October 2019, a total of 287 anaerobic digesters were operating at animal farms across the US [1, 6, 7].

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The AD process relies upon the intricate balance of various bacterial groups which carry out the four distinct phases of hydrolysis (substrate break down to simple organic and amino acids), acidogenesis (conversion of simple organic and amino acids to volatile fatty acids,  $H_2$  and  $CO_2$ ), acetogenesis (volatile fatty acids are converted to  $CH_3COOH$ ) and finally methanogenesis [8]. The microbial degradation is impacted by various factors such as *inoculum*, substrate, experimental and operational conditions [9, 10]. The anaerobic co-digestion is a process of treating more than one organic wastes in a suitable proportion and is known to improve process efficacy by balancing nutrients, diversifying microbial population and lowering toxins, balancing alkalinity, etc., and has been studied extensively [10–13]. The mono-digestion of animal manures is sometimes prone to process failures due to nutrient imbalance for the microbes owing to their low C/N ratios. The high C/N ratio of lignocellulosic feedstocks makes them feedstocks of choice as a co-substrate for AD with manures [14].

Texas is one of the major cotton (*Gossypium hirsutum*, *G. barbadense*) producing states in the US. Once picked from the farm cotton is further processed in gins to separate fiber, leaving behind cotton gin trash (CGT) as agro-industrial waste. According to Thomasson [15], ginning one bale (217–218 kg) of cotton yields about 37–147 kg of CGT [16], thus annually generating millions of tons of CGT in the states of Texas, Georgia, Arkansas, and Mississippi. The CGT, a byproduct of the cotton industry, mainly composed of clean lint (8–15%), hulls (18.5–32.9%), stem (5.2–5.9%), grass (0.1–1.1%), seed (0–2.9%), motes (20.4–21.6%), small leaves (19.4–34.9%) [17] is abundantly available in cotton-growing regions of the US. Roughly two million tons of CGT are annually produced in the US. Being a major cotton producer, 99,473 tons of CGT is produced annually in Texas [18]. The traditional management strategies have been to use it for feeding and bedding for the animals or composting, followed by addition to the soil as humus. The presence of microbial pests (verticillium wilt in particular) and pesticide residues, low heating value [19] in energy production limit the recycling options of CGT [12, 13]. However, the urgency to lower fossil fuel consumption had the scientists investigating agro-industrial wastes such as CGT as potential renewable energy sources [22]. Consequently, CGT has been investigated as a bioenergy source through ethanol production, gasification, and anaerobic degradation [20]. The high chemical oxygen demand (COD) and volatile solid (VS) contents in CGT can be recovered as biomethane through anaerobic degradation [18, 20].

Many reports indicate that the co-digestion of organic wastes with crop residues improves the biochemical methane potential (BMP) over mono-digestion [11, 23–25]. Findings from previous studies indicate that having animal

manures along with crop residues (or more than one crop residue) in an anaerobic digester meets the microbial nutritional requirements better, making the process more efficient [23, 25]. Zhang et al. [24] reported that the co-digestion of GM with wheat, rice, and corn straw yielded higher biomethane compared with GM mono-digestion.

Few studies have reported the effects of co-digesting animal manures (e.g., swine and cow manure) with cotton industry byproducts on the biomethane recovery during anaerobic digestion [23, 26, 27]. Funk et al. [27] co-digested CGT with swine manure (mixing ratios varying from 1:1–10:1) in a two-stage bioreactor. The optimum percentage for anaerobic co-digestion of different feedstocks is not universal and needs to be investigated in each case [13, 28].

The bio-solids left after the anaerobic digestion process may be of high bio-fertilizer value, depending upon the organic wastes and other digester components [29]. Nitrate, ammonium, and orthophosphate are the main plant-available forms of nitrogen and phosphorus in warm aerobic soils under normal pH conditions [30]. The effluents left after AD are well-studied for the plant available nutrients [31–33]. Kafle and Chen [34] analyzed the influents and effluents of five animal manures and compared mineral nitrogen, phosphorus, the total ammoniacal nitrogen (TAN), total nitrogen (TN), total Kjeldahl nitrogen (TKN), and total phosphate ( $PO_4^{-3}$ ). The biochemical methane potential (BMP) tests are used to determine the suitability of a given substrate for anaerobic co-digestion [4, 35]. To the best of our knowledge, no previous studies are reporting the BMP of GM–CGT co-digestion in different mixing ratios.

The objectives of this study were to investigate the BMP, biodegradability, and synergistic effect index of GM–CGT co-digestion at different mixing ratios (compared with mono-digestion of each substrate). The process was simulated utilizing the modified Gompertz equation. The liquid fraction effluents left after AD was evaluated for their bio-fertilizer quality by determining the available N and P forms.

## 2 Materials and methods

### 2.1 Chemicals, substrates, and inoculum

All the analytical grade chemicals were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA) or HACH Company (Loveland, Colorado, USA).

Goat manure was collected from the International Goat Research Center, Prairie View A&M University. It was dried at 80 °C passed through Willy mill, sieved (2 mm), and stored at 4 °C. The weathered CGT (from three months of

cotton ginning) was collected from Varisco-Court Gin Co. (5354 Steel Store Rd, Bryan, TX, 77807). The samples were collected from weathered, transition, and core layers and well-mixed to obtain a uniform gin waste. It was passed through Willy mill, sieved (2 mm) to obtain uniform-sized particles, and stored at 4 °C until further analysis. The fresh *inoculum* obtained from the Prairie View wastewater treatment plant (operating at  $36 \pm 1$  °C) was de-gassed [36–38] for a week and used for the assays.

## 2.2 Substrate characterization

The proximate analysis of well-mixed feedstocks and *inoculum* in triplicates was carried out per method 2540 [39] using a Lindberg Blue M electric furnace (Fisher Scientific, Pittsburgh, PA).

The ultimate analysis/elemental composition (C, H, N, and S) of the feedstocks was determined with the Flash 2000 elemental analyzer (Elementar Americas Inc., Ronkonkoma, NY) utilizing BBOT (C, H, N, and S; 72.5%, 6.1, 6.5 and 7.5%, respectively) as a standard compound. In compositional analysis lignin, cellulose, and hemicellulose contents were determined by thermogravimetric analysis in the PerkinElmer Diamond TG/DTG system.

For chemical analysis, triplicates of 1.0 g of each feedstock (wet weight w/w) were diluted 100 fold with deionized (DI) water in 250-mL Erlenmeyer flasks, stirred (hot plate and magnetic stir bars) for 30 min, and centrifuged (Thermo Scientific, Waltham, MA, USA) for 15 min at 5000 rpm. The pH of the supernatant was determined, appropriately diluted, and analyzed utilizing HACH kits; TNT 870, TNT 836, TNT 846, TNT 872, molybdovanadate test N tube (Method 10127), and TNT880 for alkalinity, nitrate, orthophosphate, total phosphorus, and volatile acids and total Kjeldahl nitrogen (TKN), respectively, utilizing DRB 200 reactor and DR 3900 spectrophotometer (Loveland, Colorado). The pure compounds, 500 mg/L  $\text{PO}_4$ , 1000 mg/L  $\text{CH}_3\text{COOH}$ , 25,000 mg/L  $\text{CaCO}_3$ , 500 mg/L  $\text{NO}_3^-$ , and 10 mg/L  $\text{NH}_4\text{-N}$ , were diluted as needed and used as standards to determine total phosphorus, volatile acids, alkalinity, nitrate, ammonium, and TKN, respectively. Blank was adjusted by deionized (DI) water. To equate with treatments, the triplicate concentrations (mg/L) were multiplied by 10 and reported as mean  $\pm$  SD (Table 1).

## 2.3 Biochemical methane potential tests

The effect of co-digesting GM with CGT on the biomethane recovery was determined by running BMP tests

for 62 days in 250-mL serum bottles (Fisher Scientific, Pittsburgh, PA). The tests were conducted as described by Zhang et al. [40]. There were total 36 batch reactors, having blank, GM and CGT mono-digestions and their co-digestion with varied ratios (GM/CGT; 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90) in triplicates. The organic loading rate was TS at 10%. Blank bioreactors contained all ingredients except the substrate. *Inoculum* to substrate ratio (ISR) of 0.03–0.04 (VS basis) was maintained in all mono- and co-digestions. The ISRs of 0.005, 0.007, 0.01, 0.02 and 0.04 (on VS basis) were tested on GM (unpublished data), and statistically similar yields were observed at ISRs of 0.005, 0.007, 0.01, 0.02 and 0.04. In our previous study [41], we reported that CGT yielded similar values of BMP at 0.02 and 0.03 ISRs. An equal (as that of *inoculum*) volume of trace medium [35] was added to each bioreactor. The working volume of 100 mL in each bioreactor was achieved by adding DI water. The pH of the bioreactors was in the optimum range [9]. The bioreactors were purged with  $\text{N}_2$  (99.9% purity) for five minutes, sealed with straight plug stoppers, and secured using aluminum crimp seals (Fisher Scientific, Pittsburgh, PA) for biomethane sampling and maintaining the anaerobic conditions, respectively. The bioreactors were incubated at  $36 \pm 1$  °C.

The biomethane volume was measured with one of the volumetric techniques or liquid displacement method. According to Holliger et al. [36], if the volumetric method involves absorption of  $\text{CO}_2$  in an alkaline solution, direct methane values are obtained, compositional analysis of biogas is not required. The pH of DI water was adjusted to 10:30 with 0.5 M NaOH solution and added to a large container, holding an inverted cylinder filled with the same solution. The biomethane was read by recording the initial and final volumes of the inverted graduated cylinder. The batch reactors were manually shaken once a day before collecting data to mix the contents to avoid the formation of dead zones inside [36]. The daily gas measurements were continued until the total biomethane production during three consecutive days was less than 1% of the previously accumulated [36].

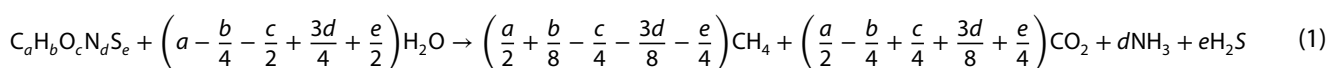
## 2.4 Theoretical maximum biomethane yield

From the chemical formula of the feedstocks (Table 1), the theoretical maximum biomethane yields (TMYs) of GM and CGT were determined by Boyle's equation [42] (Eq. 1), which is a modification of the original Buswell Muller's [43] original equation after including N and S to obtain  $\text{NH}_3$  and  $\text{SO}_2$  fractions.

**Table 1** Characteristics of goat manure (GM) and cotton gin trash (CGT)

Parameter and unit	GM	CGT	Inoculum
<i>Proximate analysis</i>			
Moisture (%)	37.7 ± 0.3	11.1 ± 0.6	97.2 ± 0.3
TS (%)	62.3 ± 0.3	88.9 ± 0.6	2.8 ± 0.3
VS (%)	52.8 ± 0.4	77.4 ± 0.2	1.5 ± 0
VS (%-TS)	84.7 ± 0.2	87.1 ± 0.8	–
Ash (%)	10.0 ± 0	11.7 ± 0.01	1.5 ± 0.4
<i>Ultimate analysis</i>			
N (%-TS)	2.8 ± 0.1	1.3 ± 0	–
C (%-TS)	43.9 ± 0.3	47.3 ± 1.2	–
H (%-TS)	1.5 ± 0.2	4.5 ± 0	–
O (%-TS)	51.3 ± 0.2	46.6 ± 1.5	–
S (%-TS)	0.6 ± 0	0.2 ± 0	–
C/N	15.7 ± 0.7	36.3 ± 0.9	–
Elemental formula	C <sub>365.9</sub> H <sub>123.0</sub> O <sub>320.4</sub> N <sub>20.2</sub> S <sub>1.9</sub>	C <sub>390.0</sub> H <sub>450.0</sub> O <sub>290.8</sub> N <sub>21.2</sub> S <sub>0.6</sub>	–
<i>Compositional analysis</i>			
Cellulose + Hemi-cellulose (%-TS)	72.4	56.0	–
Lignin (%-TS)	17.6	32.7	–
<i>Chemical properties</i>			
pH	7.9 ± 0.1	7.3 ± 0	7.5 ± 0.2
VFA (mg/L)	539.5 ± 75.7	609.5 ± 19.1	–
Alkalinity (CaCO <sub>3</sub> mg/L)	3965.0 ± 120.2	1300 ± 190.5	–
NO <sub>3</sub> <sup>−</sup> -N (mg/L)	12.7 ± 1.1	21.2 ± 0.8	–
NH <sub>4</sub> <sup>+</sup> -N (mg/L)	398.0 ± 9.9	7.9 ± 0.6	–
PO <sub>4</sub> <sup>−</sup> -(mg/L)	1230 ± 0.4	498.0 ± 6.6	–
Total N (mg/L)	429.5 ± 78.5	92.7 ± 3.5	–
NO <sub>3</sub> <sup>−</sup> + NO <sub>2</sub> <sup>−</sup> (mg/L)	14.2 ± 0.1	13.9 ± 0.2	–
TKN (mg/L)	415 ± 77.8	77.9 ± 2.1	–

All values except, nitrogen (N), carbon (C), hydrogen (H), and sulfur (S) are percentages of total wet sample weight (mean value,  $n = 3$ )



$$TMY \text{ (mL } CH_4/g_{vs}) = \frac{22400 \times \left[\left(\frac{a}{2}\right) + \left(\frac{b}{8}\right) - \left(\frac{c}{4}\right) - \left(\frac{3d}{8}\right) - \left(\frac{e}{4}\right)\right]}{12a + b + 16c + 14d + 32e} \quad (2)$$

assumed that the specific growth rate of methanogenic bacteria is directly proportional to the methane production rate in its current version as described below:

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

## 2.5 Modeling methane production

The anaerobic degradation process or bacterial growth in the bioreactors can be described by fitting the modified Gompertz equation. The original equation (based on the exponential relationship between specific growth rate and population density) was developed by Causton [44], and further modified by Gibson et al. 45 to describe bacterial growth rate in terms of exponential and lag phase duration. Lay et al. [46] further

$P(t)$  = The accumulated methane (mL/g<sub>vs</sub>, minus the blank) at digestion time  $t$  (days);  $P_0$  = Maximum cumulative methane production (mL/g<sub>vs</sub>);  $R_m$  = Maximum daily rate of biomethane production (mL/g<sub>vs</sub>/days), or specific growth rate of bacteria;  $\lambda$  = lag phase (days), minimum time to produce biomethane or time for bacterial acclimatization;  $e$  = Mathematical constant 2.718.

## 2.6 Biodegradability

The anaerobic biodegradability (BD) of a substrate is its volatile fraction that is converted to biomethane during AD. The BD of a co-digestion (at each mixing ratio) was determined using cumulative biomethane yield (EMY) from the experimental and theoretical biomethane (TMY, Eq. 1) employing Eq. (4) as described by Li et al. [47].

$$BD (\%) = \frac{EMY_{co}}{(TMY_1 \times X_1) + (TMY_2 \times X_2)} \times 100 \quad (4)$$

EMY<sub>co</sub> = The biomethane potential of co-digestion; TMY<sub>1</sub> = Theoretical biomethane potential of GM; TMY<sub>2</sub> = Theoretical biomethane potential of CGT; X<sub>1</sub> = Volatile solid fraction of GM; X<sub>2</sub> = Volatile solid fraction of CGT.

## 2.7 Synergistic effect index

The synergistic effect index (SEI) for the anaerobic co-digestions was determined as described by Li et al. [47] in equation below.

$$SEI (\%) = \frac{EMY_{co} - (EMY_1 \times X_1 + X_2 \times EMY_2)}{(X_1 \times EMY_1 + X_2 \times EMY_2)} \times 100 \quad (5)$$

EMY<sub>co</sub> = The biomethane potential of co-digestion; X<sub>1</sub> = The VS fractions of GM in the co-digestion; X<sub>2</sub> = The VS fraction of CGT in the co-digestion; EMY<sub>1</sub> = The biomethane potential of GM; EMY<sub>2</sub> = The biomethane potential of CGT.

## 2.8 Data analysis

The experimental data were processed in Microsoft Excel 2010 (Microsoft, USA). The biomethane volume was converted to dry gas volume at STP by multiplying with a dry biomethane factor of 0.838, as described by Richards et al. [48]. The blank (d-1; 20 ± 1 and d-2; 3.7 ± 0.6 mL) value was subtracted from treatments to neutralize the inoculum.

The model fit was evaluated by calculating the relative root-mean-square error (rRMSE) as described by Kafle et al. [34] utilizing Eq. 6.

$$rRMSE = \left( \frac{1}{m} \sum_{j=1}^m \left( \frac{d_j}{Y_j} \right)^2 \right)^{\frac{1}{2}} \quad (6)$$

where *m* is the number of data pairs, *j* is *j*th values, *Y* is measured methane yield (mL/g<sub>VS</sub>), and *d* is the deviations between measured and predicted methane yield.

All of the data were analyzed using the general linear model (GLM) and analysis of variance procedure of Statistical Analysis System (SAS® 9.2, SAS Institute Inc., Cary, NC, USA), and statistically significant treatment means were

separated using the least significant difference (LSD) test at 5% probability.

## 3 Results and discussion

### 3.1 Properties of goat manure and cotton gin trash

The proximate, ultimate, and compositional characteristics of feedstocks and *inoculum* are presented in (Table 1). The data values confirm that these agro wastes and *inoculum* are suitable for anaerobic degradation [9]. The C/N ratio of GM; 15.7 ± 0.7 is in the reported range of 13–20 [34, 40, 49]. The C/N ratio of CGT 36.3 ± 0.9 is also in the range of 28.0–51.3 in previous studies [20, 50, 51].

### 3.2 Daily biomethane production

The daily biomethane yields for mono- and the co-digestions of GM and CGT peaked at d-6 (Fig. 1a & b). The daily peak values of 17.6 ± 0.8, 18.9 ± 1.7, 17.3 ± 3.0, 12.8 ± 6.3, 12.5 ± 0.2, 11.8 ± 1.3, 10.2 ± 1.7, 10.7 ± 1.1, and 9.1 ± 3.7 mL/g<sub>VS</sub>, respectively, were observed on d-6 for the anaerobic digesters containing 100, 90, 80, 70, 60, 50, 40, 30, and 20% GM, respectively (Fig. 1a & b). The co-digestion with 10% GM yielded maximum daily biomethane on d-9. The maximum daily biomethane yield of 9.0 ± 0.9 mL/g<sub>VS</sub> was observed on d-13 from the CGT mono-digestion. The daily peaks signify that 6.1, 6.0, 5.2, 3.6, 4.0, 3.5, 2.9, 2.8, 2.2, 3.1, and 2.0% of the added VS was degraded in anaerobic digestions having GM and CGT mixing ratios of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, and 10:90 and 0:100. The second daily peaks of 12.3 ± 4.6 and 10.2 ± 0.8 mL/g<sub>VS</sub> were observed on d-8 and d-9 for co-digestions with 70 and 80% CGT. After the second peak, the biogas yield in all co-digestions declined steadily until 15–17 d. After 17 d, no additional peaks were observed in co-digestions. Our findings corroborate with those of Kafle and Chen [34], who reported the biomethane peak value of 18 mL/g<sub>VS</sub> at d-7 in GM during 55 d anaerobic mono-digestion. Zhang et al. [52] reported two daily peaks of 8 and 11 mL/g<sub>VS</sub> at 13 and 25 d in GM mono-digestion.

### 3.3 Cumulative biomethane production and biodegradability

The cumulative biomethane yield from mono- and co-digestions of GM and CGT at the end of BMP assays is shown in Fig. 1. The biomethane yield of 274.1 ± 7.8 mL/g<sub>VS</sub> was observed in the mono-digestion of GM. The BMP values of 261.4 ± 4.8 and 262.6 ± 4.2 mL/g<sub>VS</sub> were observed in

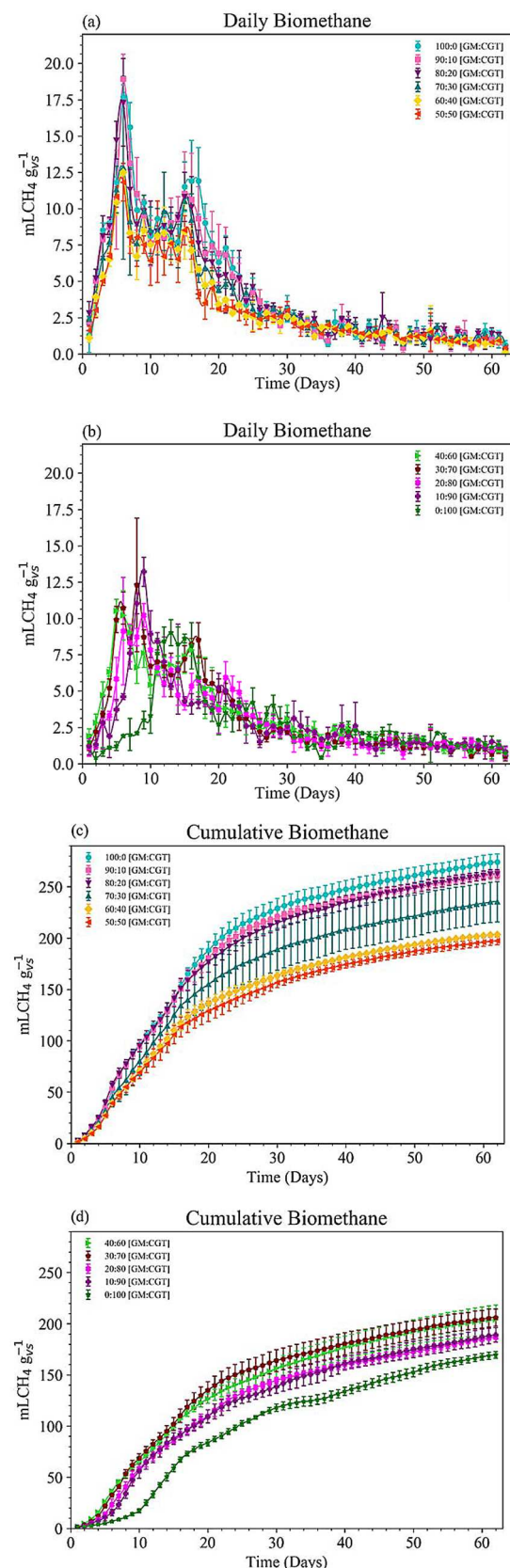


**Fig. 1** Daily biomethane yields ( $\text{mL/g}_{\text{VS}}$ ) for goat manure (GM) and cotton gin trash (CGT) mixing ratios of 90:10, 80:20, 70:30, 60:40, 50:50 (a); and 40:60, 30:70, 20:80 and 10:90 (b). The error bars represent the standard deviation. Each dot on the chart represents the mean of three values. Cumulative biomethane yields ( $\text{mL/g}_{\text{VS}}$ ) for goat manure (GM) and cotton gin trash (CGT) mixing ratios of 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 (c); and 40:60, 30:70, 20:80, 10:90 and 0:100 (d). The error bars represent the standard deviation. Each dot on the chart represents the mean of three values

GM–CGT co-digestions at the mixing ratios of 10 and 20% CGT, respectively. The co-digestion of GM and CGT with 10 and 20% CGT yielded similar ( $P < 0.0001$ ) biomethane as that of GM mono-digestion. The co-digestions having GM and CGT in proportions of 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90 yielded  $236.2 \pm 17.8$ ,  $203.5 \pm 1.1$ ,  $197.4 \pm 3.8$ ,  $204.0 \pm 14.2$ ,  $206.0 \pm 8.4$ ,  $186.7 \pm 1.9$  and  $189.0 \pm 7.1$   $\text{mL/g}_{\text{VS}}$  biomethane, respectively. As a general rule, an increase in the CGT percentage in a co-digestion significantly lowered the biomethane yield. Statistically similar ( $P < 0.0001$ ) biomethane yields of  $203.5 \pm 1.1$ ,  $197.4 \pm 3.8$ ,  $204.0 \pm 14.2$ , and  $206.0 \pm 8.4$   $\text{mL/g}_{\text{VS}}$  were observed for the co-digestion of GM with 40–70% of CGT (Fig. 1 c & d).

The technical digestion time ( $T_{80-90}$ ) is defined as the time taken to obtain 80–90% of the BMP of a given substrate [34]. An increase in CGT in co-digestion increased the  $T_{80-90}$  and was between those of the GM and CGT mono-digestions. The  $T_{80-90}$  of mono-digestions of GM and CGT was 24–39, and 41–50 d, respectively. Kafle and Chen [34] reported  $T_{80-90}$  of 31–37 d for GM mono-digestion. The  $T_{80-90}$  for the co-digestions with 10, 20, 30, 40, 50, 60, 70, 80, and 90% GM was found to be 36–47, 33–47, 30–44, 34–44, 31–43, 30–42, 30–43, 28–41, and 26–39 d, respectively.

There are no reports of anaerobic digestion of GM with CGT to date. However, previous studies have reported the biomethane potential of GM with other agricultural wastes such as wheat straw (WS), corn stalks (CS), and rice straw (RS) [40, 53] swine, and dairy cow manure [23, 27]. Zhang et al. [52] reported biomethane yields of 225.5, 261.6, 331.2, and 324.3  $\text{mL/g}_{\text{VS}}$  from 55 days of GM mono-digestion and co-digestions having missing ratios of GM/WS 30:70, GM/CS; 70:30, and GM/RS; 50:50, respectively, under mesophilic conditions. Funk et al. [27] reported that CGT and cow manure yielded maximum biomethane at mixing ratios below the value of 5:1 in a two-stage bioreactor. Macias-Corral et al. [23] stated that the co-digestion of CGT with cow manure in 5:1 ratio enhanced biomethane yield to  $87 \text{ m}^3/\text{ton}$  than the value of  $62 \text{ m}^3/\text{ton}$  in cow manure mono-digestion by utilizing the microbial diversity and nutrients in each substrate. Zhang et al. [52] reported that co-digesting GM with WS, RS, and CS improved the



biomethane over GM mono-digestion by improving the C/N ratio. The C/N ratios of GM and CGT were  $15.7 \pm 0.7$  and  $36.3 \pm 0.9$ , respectively (Table 1). The biomethane yield in the GM mono-digestion and co-digestions having 10 and 20% CGT was  $274 \pm 7.8$ ,  $261.4 \pm 4.8$ , and  $262.6 \pm 4.2$  mL/g<sub>VS</sub>, respectively, and statistically similar. The optimum C/N ratio to support anaerobic digestion for biomethane production was 15.7–19.8 in this study. Siddiqui et al. [54] reported that the optimum C/N ratio for biogas production is 15, while other researchers [55, 56] reported it to be between 16 and 25. Some studies reported the optimum value of the C/N ratio for anaerobic degradation to be in the range of 20–35 [57–59].

The biodegradability and synergistic effect index (SEI) of the anaerobic digestions are provided in Table 2. The BDs of GM and CGT mono-digestions were  $94.5 \pm 2.7$  and  $37.6 \pm 0.8\%$ , respectively. As expected, the biodegradability decreased as the proportion of CGT in the co-digestion was increased. The highest SEI of  $15.6 \pm 4.7\%$  was observed in the GM co-digestion with 70% CGT.

Goat manure and CGT used in the BMP assays have high lignin contents of 17.6 and 32.7% of TS, respectively (Table 1.). Lignin is one of the most recalcitrant components of plant-based agro-wastes and is not easily degraded during anaerobic digestion [47]. Triolo et al. [60] also stated that the lignin content of organic manures and energy crops is negatively correlated to their BMP. The acid-insoluble fraction of CGT is reported

to be 21–25% [61–63]; thus, low biodegradability of the co-digestions containing a higher proportion of CGT may be attributed to the high lignin content of the substrates. However, the EMY of  $169.6 \pm 3.5$  mL/g<sub>VS</sub> (Table 2) in CGT mono-digestion is within the range of 80–242 mL/g<sub>VS</sub> in pretreated cotton stalks reported by Adl et al. [64].

Although, Kafle and Chen [34] reported that the BMP of animal manures is negatively correlated with the manure lignin content, we report a higher BD value of  $94.5 \pm 2.7\%$ . It may be because the GM for this study was thermally treated at 80 °C, which may have further softened the lignin, thus contributing to higher biodegradability. Lignocellulosic biomass of agricultural origin is subjected to various pre-treatments such as hot water, weak alkali, or acid treatment, along with a mild increase in temperature [64–66]. These pre-treatments aim to loosen the cell walls of the recalcitrant plant components, such as lignin. In a previous study [41] we reported similar BMP values in control, hot water, and alkali pretreated CGT. The CGT in our study was collected from a pile outside the gin mill (weathered for more than 3 months). During the period it was piled up in the open, it had been exposed to environmental factors, and it was considered as a pretreatment before the BMP assays. As mentioned before the BMP of  $169.6 \pm 3.5$  mL/g<sub>VS</sub> in CGT are in the range observed by Adl et al. [64] after pretreatment of cotton residues. The storage of CGT may have enhanced BMP yield.

**Table 2** Theoretical and experimental biomethane potential, biodegradability (BD), and synergistic effect index (SEI) of goat manure (GM) and cotton gin trash (CGT) anaerobic digestions

GM/CGT	Elemental formula	C/N	TM <sup>a</sup> (mL/g <sub>VS</sub> )	EM <sup>b</sup> (mL/g <sub>VS</sub> )	BD <sup>c</sup> (%)	SEI <sup>d</sup> (%)
100:0	C <sub>365.9</sub> H <sub>123.0</sub> O <sub>320.4</sub> N <sub>20.2</sub> S <sub>1.9</sub>	15.7 ± 0.7	290.0	274.1 ± 7.8 a	94.5 ± 2.7 a	–
90:10	C <sub>368.3</sub> H <sub>155.7</sub> O <sub>317.4</sub> N <sub>20.3</sub> S <sub>1.8</sub>	17.7	313.0	261.4 ± 4.8 a	83.5 ± 1.6 b	0.9 ± 1.9 c
80:20	C <sub>370.7</sub> H <sub>188.4</sub> O <sub>314.5</sub> N <sub>20.4</sub> S <sub>1.6</sub>	19.8	332.4	262.6 ± 4.2 a	79.0 ± 1.2 c	6.5 ± 1.7 bc
70:30	C <sub>373.1</sub> H <sub>221.1</sub> O <sub>311.5</sub> N <sub>20.5</sub> S <sub>1.5</sub>	21.9	351.7	236.2 ± 17.8 b	67.1 ± 5.1 d	0.9 ± 7.6 c
60:40	C <sub>375.5</sub> H <sub>253.8</sub> O <sub>308.5</sub> N <sub>20.6</sub> S <sub>1.3</sub>	23.9	310.1	203.5 ± 1.1 c	65.7 ± 0.4 b	8.8 ± 0.6 ab
50:50	C <sub>378.0</sub> H <sub>286.5</sub> O <sub>305.6</sub> N <sub>20.7</sub> S <sub>1.3</sub>	26.0	334.5	197.4 ± 3.8 cd	59.0 ± 1.2 e	7.1 ± 2.1 bc
40:60	C <sub>380.4</sub> H <sub>319.2</sub> O <sub>302.6</sub> N <sub>9.8</sub> S <sub>1.1</sub>	28.1	357.8	204.0 ± 14.2 c	57.0 ± 4.0 ef	12.9 ± 7.9 ab
30:70	C <sub>382.8</sub> H <sub>351.9</sub> O <sub>299.7</sub> N <sub>20.9</sub> S <sub>1.0</sub>	30.1	381.5	206.0 ± 8.4 c	54.0 ± 2.2 f	15.6 ± 4.7 a
20:80	C <sub>385.2</sub> H <sub>384.6</sub> O <sub>293.2</sub> N <sub>21.0</sub> S <sub>0.9</sub>	32.2	404.6	186.7 ± 1.9 d	46.2 ± 0.5 g	6.3 ± 1.1 bc
10:90	C <sub>387.6</sub> H <sub>417.3</sub> O <sub>293.7</sub> N <sub>21.1</sub> S <sub>0.7</sub>	34.5	428.5	189.0 ± 7.1 d	44.1 ± 1.7 g	9.9 ± 4.1 ab
0:100	C <sub>365.9</sub> H <sub>123.0</sub> O <sub>320.4</sub> N <sub>20.2</sub> S <sub>1.9</sub>	36 ± 0.9	451.0	169.6 ± 3.5 e	37.6 ± 0.8 h	–
LSD <sup>e</sup>	–	–	–	14.5	4.1	7.7

Each value is a mean ± SD from triplicates. The values across a column sharing the same letter are similar at  $\alpha = 0.05$

<sup>a</sup>Theoretical biomethane potential; for pure substrates, the value was adjusted according to the VS% after initial calculation from formula (Table 1)

<sup>b</sup>Experimental biomethane potential

<sup>c</sup>Biodegradability

<sup>d</sup>Synergistic effect index

<sup>e</sup>Least significant difference

### 3.4 Modeling biomethane production

The mathematical models are integral to experimental research to optimize the process and evaluate digester performance. The models are classified as white-box, grey-box, and black box depending upon the amount of prior information required [67]. The algebraic equations (e.g., Buswell equation) which describe the biochemical reactions utilizing known information (eg chemical composition) are non-dynamic white-box models. The black-box models link input to output without requiring any knowledge of the biochemical reactions. The grey-box or mechanistically driven models require physical interpretation of the parameters by estimation, which is adjustable. Most of the dynamic models describing anaerobic digestion are in this category [67]. In this study, the anaerobic digestion process was optimized and evaluated by the non-dynamic white-box model; Boyle's equation (Eq. 1), and by fitting the cumulative data to the modified Gompertz equation (Eq. 3.). The modified Gompertz model was validated by calculating the coefficient of determination ( $R^2$ ) and rRMSE. The parameters obtained by fitting the data are given in Table 3. The predicted, and experimental biomethane values were similar as described in Fig. 2. The 20, 50, and 60% additions of CGT (to GM) in co-digestion significantly lowered the lag phase compared to GM mono-digestion. These observations are consistent with the feedstock properties, with GM having a higher proportion of soluble/readily available fraction of volatile solids for microbial consumption while CGT provided carbon (being

a high C/N ratio of the two substrates). The maximum and similar daily rates of biomethane production ( $R_m$ ) were observed in GM mono-digestion and a 10% co-digestion with CGT. As the proportion of CGT in a co-digestion rose, the maximum daily rate decreased. In the co-digestions of 10, 20, 40, and 50% CGT, the  $R_m$  values were 5.9, 6.1, 6.4, and 6.7 (Table 3.), respectively, and statistically similar ( $P < 0.0001$ ). The high  $R^2$  values of 0.986–0.990 prove that the modified Gompertz equation fits very well with the cumulative data and hence the anaerobic digestion of GM–CGT. The least  $R^2$  value of 0.986 was observed in CGT mono-digestion and GM co-digestion with 10% CGT. The rRMSE values varied between 0.487 and 1.663. Similar rRMSE values for observed and modified Gompertz model predicted biomethane from the anaerobic digestion of organic wastes had earlier been reported by Srivastava et al. [68].

### 3.5 Phosphate and nitrate in liquid fraction of effluents

The  $\text{PO}_4^-$  concentration in the liquid fraction of the animal manure digestates varies between 110–125 mg/L [69]. The observed  $\text{PO}_4^-$  concentration of digestate from GM's mono-digestion was  $178.7 \pm 37.2$  mg/L. However, the  $\text{PO}_4^-$  and  $\text{NO}_3^-$  concentration in the liquid fraction of the digestate (Table 4) was lower than feedstocks in all treatments. The concentrations were in proportion to the quantity of feedstocks in each mixing ratio. The animal manures are known to have high organic N and P contents,

**Table 3** Gompertz parameters and experimental biomethane from goat manure (GM) and cotton gin trash (CGT) anaerobic digestions

GM: CGT	$P^a$ (mL/g <sub>vs</sub> )	$E^b$ (mL/g <sub>vs</sub> )	$R_m^c$ (mL/g <sub>vs</sub> )	$\lambda^d$ (d)	$R^2$	rRMSE
100:0	263.7 ± 7.9 a	274.1 ± 7.8 a	10.6 ± 0.7 a	1.4 ± 0.4 bc	0.993 ± 0.002 a	1.280
90:10	252.3 ± 2.8 a	261.4 ± 4.8 a	10.2 ± 0.4 ab	1.2 ± 0.1 bc	0.993 ± 0.002 a	0.608
80:20	252.6 ± 3.9 a	262.6 ± 4.2 a	9.5 ± 0.6 b	0.5 ± 0.1 d	0.992 ± 0.003 ab	0.863
70:30	226.7 ± 16.7 b	236.2 ± 17.8 b	8.3 ± 1.3 c	0.8 ± 0.2 cd	0.991 ± 0 ab	0.487
60:40	196.0 ± 0.9 c	203.5 ± 1.1 c	7.3 ± 0.5 cd	0.7 ± 0.3 cd	0.990 ± 0.001 abc	1.663
50:50	190.2 ± 1.4 cd	197.4 ± 3.8 cd	6.7 ± 0.6 de	0.3 ± 0.2 d	0.988 ± 0.004 bc	1.142
40:60	198.1 ± 14.8 c	204.0 ± 14.2 c	6.4 ± 0.3 de	0.3 ± 0.4 d	0.990 ± 0.004 abc	1.009
30:70	196.8 ± 8.8 c	206.0 ± 8.4 c	7.4 ± 0.7 cd	1.4 ± 0.4 bc	0.989 ± 0.005 abc	1.363
20:80	178.5 ± 4.9 d	186.7 ± 1.9 d	6.1 ± 0.3 e	1.7 ± 0.6 b	0.990 ± 0.001 abc	1.373
10:90	181.4 ± 7.5 d	189.0 ± 7.1 d	5.9 ± 0.4 ef	1.6 ± 0.7 b	0.986 ± 0.002 c	1.452
0:100	163.5 ± 3.6 e	169.6 ± 3.5 e	5.1 ± 0.3 f	4.8 ± 0.7 a	0.986 ± 0.003 c	0.502
LSD <sup>e</sup>	14.3	14.5	1.0	0.7	0.005	–

Each value is a mean ± SD from triplicates. The values across a column sharing the same letter are similar at  $\alpha = 0.05$

<sup>a</sup>Simulated biomethane potential

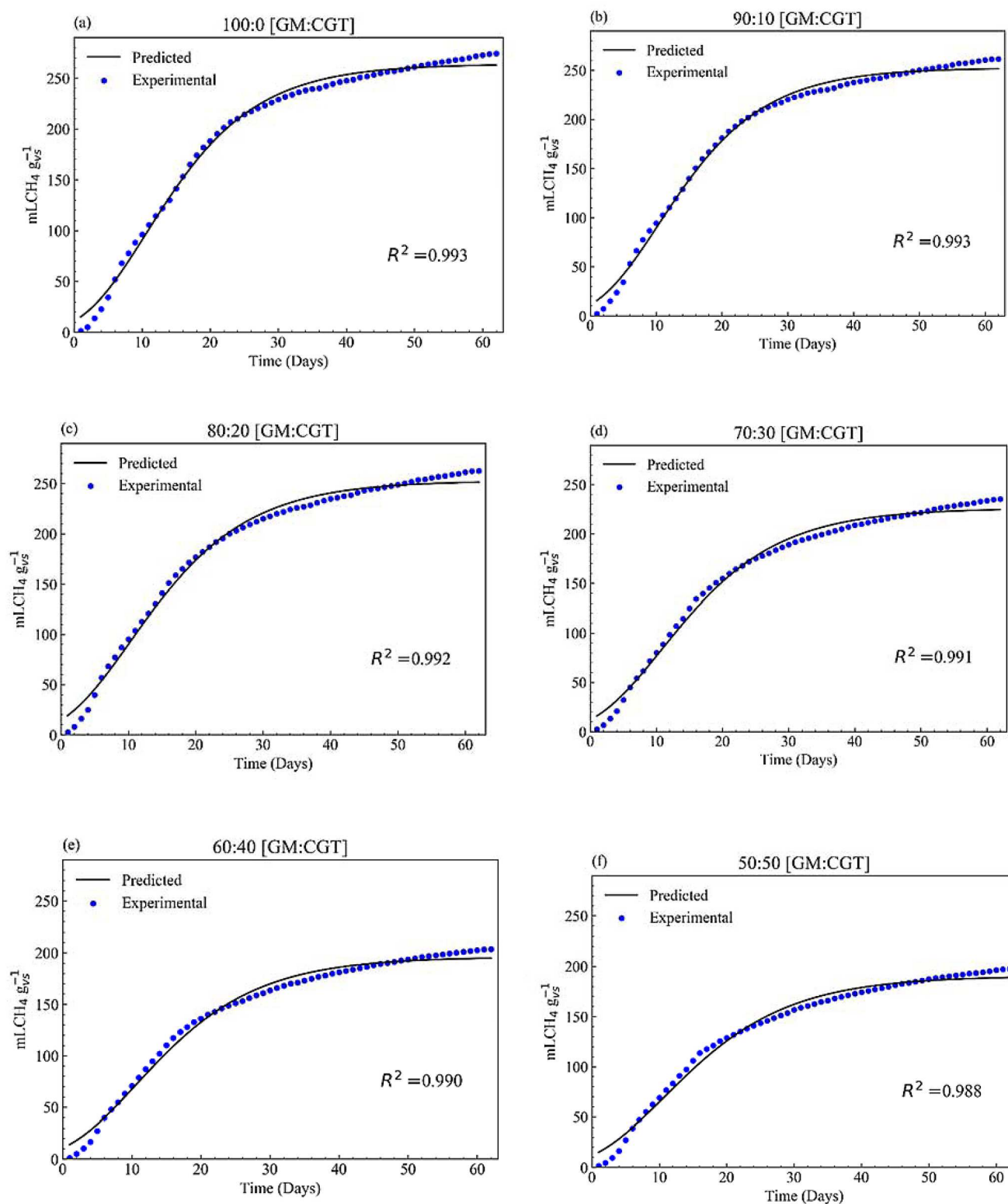
<sup>b</sup>Experimental biomethane potential

<sup>c</sup>Maximum daily rate of biomethane production

<sup>d</sup>Lag phase

<sup>e</sup>Least significant difference





**Fig. 2** The modified Gompertz model-simulated and experimental biomethane yields ( $\text{mL/g}_{\text{VS}}$ ) in co-digestions having different proportions of goat manure (GM) and cotton gin trash (CGT)

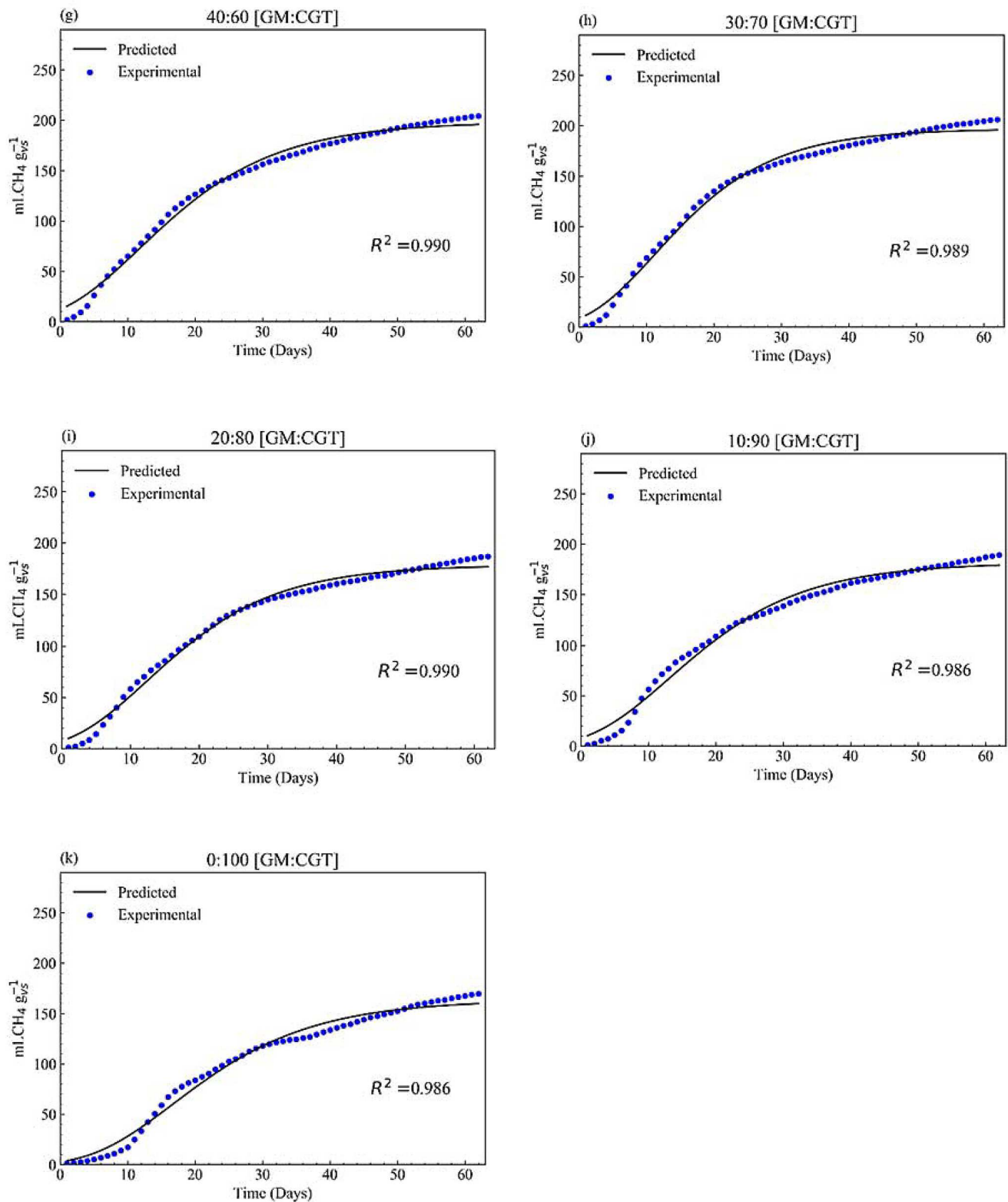


Fig. 2 (continued)

**Table 4** Phosphate ( $\text{PO}_4^{3-}\text{-P}$ ) and nitrate ( $\text{NO}_3^{--}\text{-N}$ ) concentrations in the liquid fraction of digestate from goat manure (GM) and cotton gin trash (CGT) mono and co-digestions

GM/CGT	$\text{PO}_4^{3-}\text{-P}$ (mg/L)	$\text{NO}_3^{--}\text{-N}$ (mg/L)
100:0	178.7 ± 37.2 ab	9.4 ± 0.0 a
90:10	198.0 ± 41.5 a	6.5 ± 2.7 b
80:20	160.0 ± 10.0 ab	7.1 ± 1.3 b
70:30	161.0 ± 4.4 ab	7.0 ± 0.1 b
60:40	154.7 ± 12.3 ab	6.0 ± 1.8 bc
50:50	153.7 ± 13.8 b	3.8 ± 1.4 d
40:60	142.3 ± 24.0 bc	2.8 ± 0.3d
30:70	106.0 ± 1.7 c	3.9 ± 0.2 cd
20:80	101.1 ± 51.2 c	2.9 ± 0.6 d
10:90	45.8 ± 3.6 d	2.5 ± 0.4 d
0:100	26.1 ± 4.7 d	2.8 ± 0.3 d
LSD	44.0	2.1

Each value is a mean ± SD from triplicates. The values across a column sharing the same letter are similar at  $\alpha=0.05$

which usually are not in the available forms but during the anaerobic degradation process, the unavailable forms of N and P are solubilized to available forms by the microbial populations carrying out the anaerobic digestion process [70]. The availability of nutrients in the digestate and animal slurries depends upon their pH which is affected by the concentration of various ions (released during organic matter degradation or added in the form of micronutrients) in the solution [29]. The pH of the digestates was between 6.8 and 7.0. Moller and Sommer [71] reported that most of the phosphorus and organic nitrogen stays in the solid fraction of the digestate, while the liquid fraction contains most of the inorganic nitrogen. The inorganic nitrogen may be in the  $\text{NH}_4^+$  form, while  $\text{PO}_4^{3-}$  may be in the solid fraction of the digestate.

## 4 Conclusion

This research was aimed at improving the performance of digesters at goat CAFOs by incorporating cotton gin trash; another agro-waste. The GM and CGT were anaerobically digested, the process performance was optimized and evaluated utilizing suitable models. Despite high non-degradable contents, the GM co-digestion with 10 and 20% CGT yielded statistically similar biomethane as that of GM. The high experimental biomethane yield (94% of the theoretical value) in GM mono-digestion indicates high performance without process inhibition, which might be aided by its thermal treatment. The farms operating GM mono-digestions may incorporate CGT (10–20%) and mitigate the twin problems of environmental pollution from GM and CGT disposal at goat farms and cotton gins,

respectively. Although CGT incorporation did not improve biomethane yield over GM mono-digestions, the values observed were in the range reported by other researchers after pretreatment of cotton residues. The liquid fraction of the digestate had negligible nitrate and phosphate concentrations. Thus, this study may provide valuable information to the researchers in this field as there are no reports investigating these feedstocks together. Future studies will focus on pre-treatments to soften the lignin content of the CGT before co-digesting with GM and evaluating effluents on crops as potential biofertilizers.

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## Declarations

**Conflict of interest** The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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