

## EFFECT OF BUFFALO-TO-COW MANURE MIXING RATIO ON METHANE YIELD AND PRODUCTION KINETICS DURING MESOPHILIC ANAEROBIC CO-DIGESTION

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### Abstract

Karachi, Pakistan, faces a chronic natural-gas shortage while its cattle colonies generate substantial quantities of buffalo manure (BM) and cow manure (CM). This study evaluated the effect of BM:CM mixing ratio on methane yield and production kinetics under mesophilic batch digestion. BM and CM were digested alone and in five mixtures (BM:CM = 80:20 to 20:80, VS basis) at 37 °C for 62 days. Kinetics were described using the modified Gompertz model and mixture performance was compared with VS-weighted theoretical yields. BM showed a shorter fitted lag phase than CM (5.2 vs 14.4 d), whereas CM had the higher mean final yield (133 ± 43 vs 109 ± 26 mL CH<sub>4</sub>/g VS added). Mixtures containing 40-80% BM increased the fitted maximum methane-production rate from 3.3-4.0 to 4.7-6.5 mL/g VS/d, with the highest rate at 50:50, and reached 80% of their measured final yield 9-17 days sooner than either manure alone. Four mixtures showed apparent positive deviations of 14-23% from weighted theoretical yields; however, final-yield differences were not statistically significant (ANOVA,  $p = 0.77$ ), and uncertainty in the interaction index did not establish biological synergy. The 20:80 result was sensitive to two anomalously high late measurements. Overall, co-digestion primarily accelerated methane-production kinetics rather than demonstrating a significant increase in ultimate methane yield. These results support further evaluation of flexible BM-CM blending in better-replicated and semi-continuous digestion systems.

### INTRODUCTION

Natural gas has long been the backbone of Pakistan's energy system. Including imported liquefied natural gas (LNG), it supplies more than 60% of the country's energy and power needs (Masood et al., 2023). Domestic reserves are being depleted. Indigenous production is projected to fall from about 4 to 2 billion cubic feet per day,

while demand is expected to grow by about 1.5 billion cubic feet per day (Lund et al., 2023). The widening gap is filled with imported LNG, which exposes the economy to volatile international prices and drains foreign exchange; petroleum-group imports already account for roughly 30% of national import payments (Syed Muhammad Mehdi, 2025). For consumers, the shortfall

appears as recurrent gas and electricity load-shedding, with losses that spread across every sector of the economy (Rani et al., 2023)

Karachi, Pakistan's largest city and its main industrial and commercial centre, is particularly exposed to the national energy-supply challenge. Households, industry, compressed natural gas stations and power plants compete for constrained gas supplies, while the city also experiences persistent electricity outages (Haider, 2025). A local renewable source of methane that can replace part of this demand, either as biogas for cooking, heating and power or as biomethane upgraded to pipeline quality, could therefore reduce dependence on depleting domestic gas and imported LNG.

Biomethane produced by anaerobic digestion (AD) of organic wastes is interchangeable with natural gas. At the global scale, its technical potential is equivalent to 29% of natural gas use and two-thirds of net natural gas imports (Marconi & Rosa, 2023). Livestock manure is usually the largest single feedstock. Cattle manure, for example, accounts for 73-84% of the biomethane potential of the state of Rio de Janeiro, where upgraded biogas could meet 10-22% of natural gas demand (Oliveira et al., 2024). In Iran, manure-derived biomethane could cover about 4% of national gas demand (Vaysi et al., 2024). Pakistan produces more than 840 million tonnes of animal manure each year, enough to generate about 28 million m<sup>3</sup> of biogas per day (Wakeel et al., 2023). Yet most of this resource is wasted. Buffalo dung is commonly dried and burned as household fuel without treatment (Korai et al., 2024), and uncontrolled storage can lose up to two-thirds of the energy value of manure while emitting methane to the atmosphere (Mazurkiewicz, 2023). Developing biogas from manure has been identified as a direct way to save foreign exchange spent on imported fuels (Syed Muhammad Mehdi, 2025) and as a mature route to renewable gas (Mignogna et al., 2023).

Karachi is unusual in that a substantial livestock-manure resource is generated within and around the city. The cattle colonies of Landhi and Surjani towns keep buffaloes and cows at high density to

supply the city with milk. Their manure is generated close to potential energy consumers but is commonly handled as a waste stream. Converting this manure into biogas could therefore contribute simultaneously to renewable-energy generation and improved manure management. Because buffalo manure (BM) and cow manure (CM) are produced side by side in the same colonies, a digester operating in this setting is likely to receive mixtures of the two; consequently, their co-digestion behaviour warrants investigation.

Co-digestion of two or more substrates can improve nutrient balance, buffering capacity and process stability compared with mono-digestion (Mignogna et al., 2023). Whether the substrates interact synergistically depends strongly on the pair. In a meta-analysis of 432 data sets, (Chuenchart et al., 2024) found pronounced synergy when manures were co-digested with lignocellulosic biomass or food waste. Synergy indices for mixtures of manures with other wastes are usually more modest, for example 1.10-1.18 for slaughterhouse waste co-digested with cattle or pig manure (Jo et al., 2024). Synergy has also been reported between two manures, cow and sheep manure (Li et al., 2020). The modified Gompertz model is widely used to describe how co-digestion changes the lag phase and the maximum rate of methane production (Ulukardesler, 2023). Such changes can matter as much as the final yield, because they determine the retention time, and hence the size, of a digester (Yu et al., 2024).

In Pakistan, buffalo dung has been co-digested with pretreated crop residues (Noonari et al., 2023), petroleum sludge (Korai et al., 2024), fruit and vegetable waste (Kandhro et al., 2025) and poultry manure (Iqbal Jarwar et al., n.d.), and its digestion in full-scale dome digesters has been documented (Kandhro et al., 2024). Elsewhere, buffalo manure has been co-digested with excess sludge (Zhou et al., 2023). However, we found no study of buffalo manure co-digested with cow manure, the combination that is actually available in Karachi's cattle colonies. The methane potential, production kinetics and interactions of their mixtures are therefore unknown.

This study was designed as a first step towards understanding the co-digestion behaviour of buffalo and cow manure generated in Karachi's cattle colonies. Its objectives were to (i) determine the biochemical methane potential and production kinetics of BM and CM digested alone; (ii) determine how the BM:CM ratio affects methane yield and kinetics in mesophilic batch co-digestion, using the modified Gompertz model; and (iii) quantify departures from additive methane production between the two manures and assess whether broad mixing-ratio flexibility is technically plausible.

## 2. Materials and methods

### 2.1. Substrates and inoculum

Fresh BM and CM were collected from a cattle farm in the Cattle Colony (Bhains Colony), Landhi Town, Karachi, Sindh, Pakistan (Fig. 1), on a single sampling occasion. The inoculum was digestate taken from a full-scale biogas plant in Landhi fed with buffalo and cow manure. All substrates and the inoculum were stored at 4 °C until use. Before the assays, the inoculum was pre-incubated at 37 °C for 10 days to reduce endogenous methane production. The characteristics of the substrates and inoculum are given in Table 1.

Table 1. Characteristics of the substrates and inoculum (mean  $\pm$  SD, n = 3).

| Material                   | pH              | Moisture (%)     | TS (%)           | VS (% TS)        |
|----------------------------|-----------------|------------------|------------------|------------------|
| Buffalo manure (BM)        | 6.87 $\pm$ 0.31 | 81.73 $\pm$ 0.78 | 18.32 $\pm$ 3.96 | 78.02 $\pm$ 0.93 |
| Cow manure (CM)            | 6.04 $\pm$ 0.25 | 78.63 $\pm$ 0.50 | 19.48 $\pm$ 2.52 | 81.99 $\pm$ 2.31 |
| Inoculum (digested manure) | 7.27 $\pm$ 0.16 | 91.09 $\pm$ 0.17 | 8.91 $\pm$ 1.76  | 78.26 $\pm$ 1.02 |

TS = total solids; VS = volatile solids, expressed as percentage of TS. Small differences between moisture and TS totals reflect rounding of independently reported mean values.

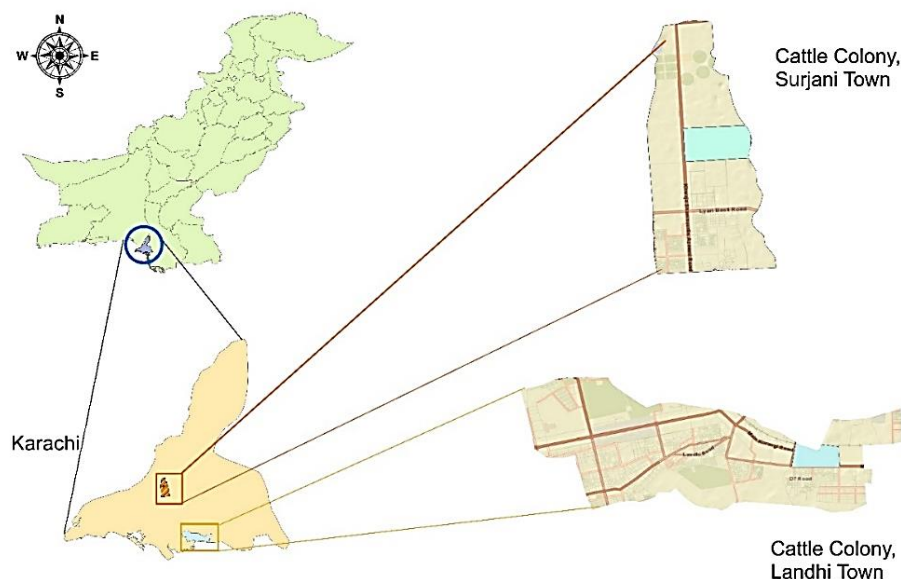


Fig. 1. Location of the cattle colonies in Surjani Town and Landhi Town, Karachi, Pakistan. Manure and inoculum were collected in Landhi Town.

## 2.2. Analytical methods

Moisture content, total solids (TS) and volatile solids (VS) were determined according to Standard Methods 2540 B and 2540 E (APHA (2005) Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC. - References - Scientific Research Publishing, n.d.): TS by drying at 103-105 °C for 24 h, and VS by ignition of the dried residue at 550 °C for 2 h. The pH was measured electrometrically following USEPA Method 9045D (US EPA, n.d.) in the sample supernatant using a Lovibond SensoDirect 150 pH meter. All analyses were performed in triplicate.

## 2.3. Experimental design and BMP assays

Biochemical methane potential (BMP) assays followed general recommendations for batch BMP tests (Holliger et al., 2016). BM and CM were digested alone and in five mixtures with BM:CM ratios of 80:20, 60:40, 50:50, 40:60 and 20:80 on a VS basis (Table 2). Reactors containing inoculum only were run as blanks to correct for endogenous methane production. Each reactor was a 500 mL borosilicate glass bottle and received 20 g VS of substrate at an inoculum-to-substrate ratio (ISR) of 1 on a VS basis. Bottles were sealed with silicone stoppers, flushed with nitrogen for 5 min, and incubated in a temperature-controlled water bath at  $37 \pm 2$  °C for 62 days, until daily production approached blank levels. BM, CM and the 50:50 mixture were run in triplicate, and the blank and the 80:20, 40:60 and 20:80 mixtures in duplicate.

Table 2. Composition of the mono- and co-digestion reactors (per reactor).

| Reactor               | BM<br>(% VS) | CM<br>(% VS) | BM wet<br>mass (g) | CM wet<br>mass (g) | BM<br>(g) | VS<br>(g) | CM<br>(g) | VS<br>(g) | n |
|-----------------------|--------------|--------------|--------------------|--------------------|-----------|-----------|-----------|-----------|---|
| Blank (inoculum only) | 0            | 0            | 0                  | 0                  | 0         | 0         | 0         | 0         | 3 |
| BM                    | 100          | 0            | 138.8              | 0                  | 20        | 0         | 0         | 0         | 3 |
| CM                    | 0            | 100          | 0                  | 117.0              | 0         | 20        | 20        | 0         | 3 |
| BM:CM 80:20           | 80           | 20           | 111.0              | 23.4               | 16        | 4         | 4         | 16        | 3 |
| BM:CM 60:40           | 60           | 40           | 83.3               | 46.8               | 12        | 8         | 8         | 12        | 3 |
| BM:CM 50:50           | 50           | 50           | 69.4               | 58.5               | 10        | 10        | 10        | 10        | 3 |
| BM:CM 40:60           | 40           | 60           | 55.5               | 70.1               | 8         | 12        | 12        | 8         | 3 |
| BM:CM 20:80           | 20           | 80           | 27.8               | 93.6               | 4         | 16        | 16        | 4         | 3 |

BM = buffalo manure; CM = cow manure; VS = volatile solids; n = number of replicate reactors. All reactors received inoculum at an ISR of 1 (VS basis).

## 2.4. Biogas collection and methane measurement

The set-up is shown in Fig. 2. Biogas from each reactor was led through Tygon tubing into a 100 mL CO<sub>2</sub>-absorption bottle containing 80 mL of 3 M NaOH with 5 mL of 0.4% thymolphthalein as a pH indicator, which signals exhaustion of the absorbent. The absorption bottles were closed with rubber stoppers fitted with inlet and outlet

tubes. CO<sub>2</sub> (and H<sub>2</sub>S) was retained in the alkaline solution, and the remaining methane was collected in gas bags and quantified daily with a gas-tight syringe (Filer et al., 2019). Because the source record does not document a temperature-and-pressure normalization procedure, methane volumes are reported here as measured mL rather than normalized mL.

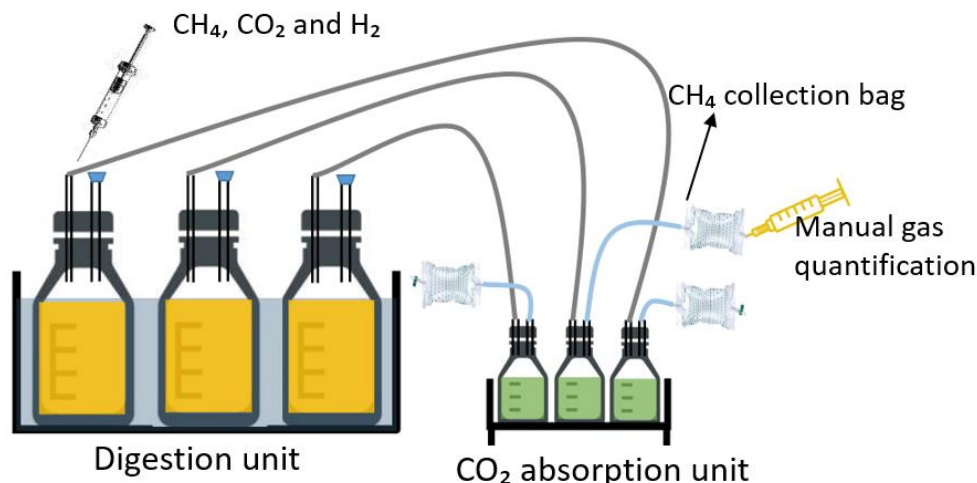


Fig. 2. Schematic of the manual BMP set-up: digestion unit in a temperature-controlled water bath, CO<sub>2</sub> absorption unit (3 M NaOH), and methane collection bags with manual syringe quantification.

## 2.5. Data analysis

The methane yield of each reactor was calculated per gram of VS added, after subtracting the mean methane production of the blanks (Eq. (1)), as recommended for BMP tests (Holliger et al., 2016):

$$\text{BMP} = \frac{(V_{\text{CH}_4, \text{sub+ino}} - V_{\text{CH}_4, \text{ino}}) / m_{\text{VS, sub}}}{(1)}$$

where  $V_{\text{CH}_4, \text{sub+ino}}$  is the cumulative methane volume from a reactor containing substrate and inoculum (mL),  $V_{\text{CH}_4, \text{ino}}$  is the mean cumulative methane volume from the blanks (mL), and  $m_{\text{VS, sub}}$  is the mass of substrate VS added (g).

VS reduction was calculated with Eq. (2). To allow comparison with studies that report yields per unit of VS degraded, the specific methane yield per gram of VS removed (SMY) was also calculated (Eq. (3)):

$$\text{VS reduction (\%)} = \frac{(V_{\text{VS consumed}} / V_{\text{VS initial}}) \times 100}{(2)}$$

$$\text{SMY} = \frac{\text{BMP}}{(\text{VS reduction} / 100)} \quad (3)$$

where  $V_{\text{VS consumed}}$  is the VS removed by the end of the assay and  $V_{\text{VS initial}}$  is the VS present at the start. VS-reduction values are used as reported in the experimental dataset; because replicate-level VS-removal measurements were not documented

in the source manuscript, they are interpreted descriptively.

Interactions between the two manures were evaluated with the synergy index  $\alpha$  (Eq. (4)). The theoretical yield of each mixture is the VS-weighted average of the yields of the individual manures (Eq. (5)) (Raposo et al., 2011; Soomro et al., 2020):

$$\alpha = \frac{Y_{\text{experimental}}}{Y_{\text{theoretical}}} \quad (4)$$

$$Y_{\text{theoretical}} = Y_{\text{BM}} \times f_{\text{BM}} + Y_{\text{CM}} \times f_{\text{CM}} \quad (5)$$

where  $Y_{\text{BM}}$  and  $Y_{\text{CM}}$  are the mean yields of BM and CM in mono-digestion and  $f_{\text{BM}}$  and  $f_{\text{CM}}$  are their VS fractions in the mixture.  $\alpha$  was calculated on both the VS-added and VS-removed bases. Values above, equal to, or below 1 indicate positive, additive, or negative departures, respectively, from the yield predicted by the weighted mono-digestion values. Because replicate variability was substantial,  $\alpha$  values were interpreted together with their uncertainty rather than as proof of biological synergy.

## 2.6. Kinetic modelling: modified Gompertz model

In batch anaerobic digestion, cumulative methane production typically follows a sigmoidal curve with three phases. Production is slow at first while the microbial community adapts and hydrolysis

products accumulate (lag phase). It then reaches a maximum rate, and finally levels off as the degradable substrate is exhausted. The modified Gompertz model describes this pattern with three parameters that have a direct physical meaning. It was reparameterised by (Zwietering et al., 1990) for microbial growth curves and has since been widely applied to methane production in anaerobic digestion, including the co-digestion of manures (Lee et al., 2024; Saha et al., 2025; Ulukardesler, 2023). In its usual form for methane production it is written as Eq. (6):

$$B(t) = P \cdot \exp\{-\exp[(R_m \cdot e / P)(\lambda - t) + 1]\} \quad (6)$$

where  $B(t)$  is the cumulative methane yield at digestion time  $t$  (mL CH<sub>4</sub>/g VS added);  $P$  is the methane production potential, i.e. the yield approached as  $t \rightarrow \infty$  (mL CH<sub>4</sub>/g VS added);  $R_m$  is the maximum methane production rate (mL CH<sub>4</sub>/g VS added/d);  $\lambda$  is the lag phase (d);  $t$  is the digestion time (d); and  $e = \exp(1) = 2.718$ .

Geometrically,  $R_m$  is the slope of the curve at its inflection point.  $\lambda$  is the time at which the tangent drawn at the inflection point crosses the time axis, so it measures the delay before methane production proceeds at a substantial rate. The time at which the maximum rate occurs (the inflection point,  $t_i$ ) follows from Eq. (6) as:

$$t_i = \lambda + P / (R_m \cdot e) \quad (7)$$

at which point about 37% ( $1/e$ ) of the methane potential has been produced. The technical digestion time ( $T_{80}$ ) was defined as the time required to reach 80% of the measured final yield and was read directly from the mean cumulative curve (Kafle & Chen, 2016).

For each treatment, Eq. (6) was fitted to the blank-corrected mean cumulative methane yield over days 1-62 by non-linear least squares, using the trust-region reflective algorithm (scipy.optimize.curve\_fit, Python). All three parameters were constrained to be non-negative. The initial estimates were  $P$  equal to the measured final yield,  $R_m = 5$  mL/g VS/d and  $\lambda = 8$  d. Standard errors (SE) of the parameters were obtained from the covariance matrix of the fit. Because the fits were made to mean curves, these

SE describe model-fit uncertainty only; variability between replicate reactors is reported separately through the measured yields (mean  $\pm$  SD).

Goodness of fit was evaluated with the coefficient of determination ( $R^2$ ) and the root-mean-square error (RMSE, Eq. (8)). The agreement between the predicted potential and the measured yield at the end of the assay was evaluated with Eq. (9):

$$RMSE = \left[ \frac{1}{n} \sum (B_{meas,i} - B_{pred,i})^2 \right]^{1/2} \quad (8)$$

$$\text{Difference (\%)} = \frac{(P - B_{meas,62})}{B_{meas,62}} \times 100 \quad (9)$$

where  $B_{meas,i}$  and  $B_{pred,i}$  are the measured and predicted cumulative yields on day  $i$ ,  $n$  is the number of observations (62), and  $B_{meas,62}$  is the measured yield on day 62. Fits with  $R^2 \geq 0.98$  and an absolute difference of less than 10% were taken to describe the data adequately, in line with the prediction-error criterion used in comparable kinetic studies (Hakimi et al., 2023). A larger difference indicates that the curve had not levelled off by the end of the assay, in which case  $P$  is an extrapolation.

## 2.7. Statistical analysis

Methane yields are reported as mean  $\pm$  standard deviation (SD) of the replicate reactors. Differences in BMP among treatments with at least three replicates were tested by one-way ANOVA followed by Tukey's HSD test, at a significance level of  $p < 0.05$ . The standard error of  $\alpha$  was obtained by propagating the standard errors of the experimental and theoretical yields.

## 3. Results and discussion

### 3.1. Characteristics of substrates and inoculum

Both manures had high moisture contents (78.6-81.7%) and similar TS contents (18.3-19.5%), with VS accounting for 78.0% and 82.0% of TS in BM and CM, respectively (Table 1). The two manures are therefore broadly similar in gross composition. The main difference was pH: CM was more acidic (6.04) than BM (6.87), while the inoculum was near-neutral (7.27). The TS content of the inoculum (8.91%) was about half that of the fresh manures, as expected for a digestate whose readily degradable organic matter had already

been converted to biogas. Fibre fractions and the C/N ratio were not determined.

### 3.2. Methane production from mono-digestion

Both manures produced a small methane peak on day 2 ( $\approx 138$  mL/d for BM and  $\approx 77$  mL/d for CM; Fig. 3a), probably reflecting readily degradable organics. This was followed by a period of low production lasting until about day 10 for BM and day 15 for CM. The lag coincided with relatively low initial reactor pH (6.0-6.4; Table 3), suggesting that initial acid-base conditions may have contributed to delayed methanogenic activity. BM then entered its main production phase, with maxima of  $\approx 150$  mL/d between days 13 and 16. CM produced methane over a longer period, from about day 20 to day 45, with maxima of  $\approx 160$  mL/d on day 23 and  $\approx 130$  mL/d on day 32. Consequently, cumulative production from BM exceeded that from CM until about day 35, after which CM overtook it (Fig. 3b).

The modified Gompertz model described the mean cumulative curves well ( $R^2 = 0.991$  and  $0.998$ ; Fig. 5a; Table 4; Section 3.4). It indicated a shorter fitted lag for BM than CM ( $\lambda = 5.2$  vs  $14.4$  d), while CM had a slightly higher maximum rate ( $R_m = 4.0$  vs  $3.3$  mL/g VS/d) and a higher fitted potential. The measured methane yield after 62 days was  $109 \pm 26$  mL CH<sub>4</sub>/g VS added for BM and  $133 \pm 43$  mL CH<sub>4</sub>/g VS added for CM. CM

also showed the higher reported VS reduction (49% vs 45%; Table 3). Thus, BM exhibited earlier methane-production kinetics, whereas CM showed the higher mean final yield.

These yields lie at the lower end of the range reported for bovine manures. (Kafle & Chen, 2016) measured 204 mL CH<sub>4</sub>/g VS and 58.6% VS removal for dairy manure, and (Basumatary et al., 2024) and (Kandhro et al., 2025) reported batch yields of about 250-260 mL CH<sub>4</sub>/g VS for co-digestion mixtures rich in cattle and buffalo dung. For buffalo dung, full-scale dome digesters in Pakistan produced 0.23-0.38 m<sup>3</sup> biogas/kg VS added (67-71% CH<sub>4</sub>), depending on season (Kandhro et al., 2025), which corresponds to roughly 155-270 mL CH<sub>4</sub>/g VS. Two features of the method probably contributed to the lower yields. The substrate loading was high (50 g VS/L at an ISR of 1). Low ISRs reduce measured methane yields: for silage co-digested with cow manure, yields at an ISR of 1 were only about half of those at an ISR of 4 (Sarode et al., 2023). Higher organic loading also lengthens the lag phase and slows hydrolysis (Aili Hamzah et al., 2023), consistent with the low initial pH and the lag observed here. The blank was also relatively large ( $\approx 1,060$  mL, about one-third of the gross production of the BM reactors), which magnifies the effect of any error in the blank correction.

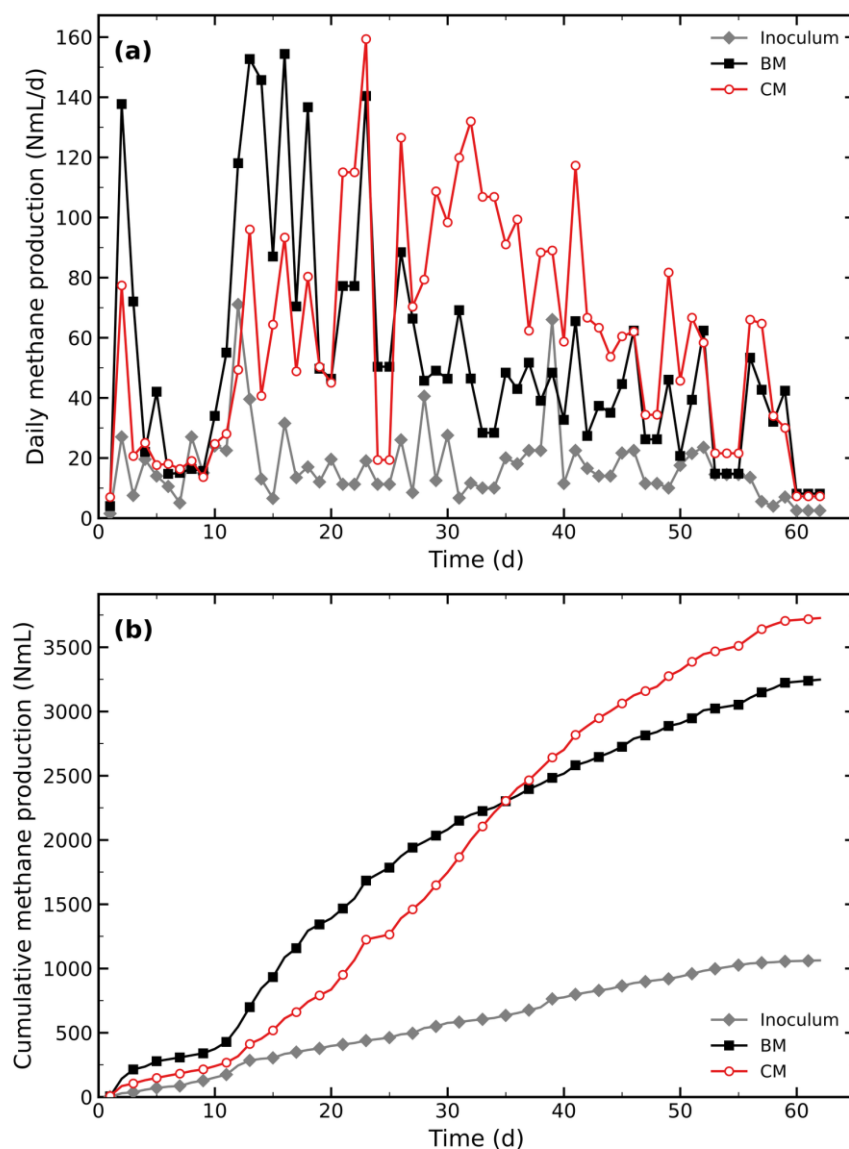


Fig. 3. (a) Daily and (b) cumulative methane production during mono-digestion of buffalo manure (BM) and cow manure (CM), and from the inoculum-only blank, at 37 °C. Values are means of replicate reactors (BM and CM, n = 3; blank, n = 2).

Table 3. pH and VS reduction in the mono- and co-digestion reactors.

| Reactor               | Initial pH | Final pH (mean) | VS reduction (%) |
|-----------------------|------------|-----------------|------------------|
| Blank (inoculum only) | 7.23       | 6.85            | 28               |
| BM                    | 6.35       | 7.14            | 45               |
| CM                    | 6.04       | 7.04            | 49               |
| BM:CM 80:20           | 6.38       | 7.08            | 55               |
| BM:CM 60:40           | 6.00       | 7.20            | 58               |

| Reactor     | Initial pH | Final pH (mean) | VS reduction (%) |
|-------------|------------|-----------------|------------------|
| BM:CM 50:50 | 6.10       | 7.18            | 63               |
| BM:CM 40:60 | 6.28       | 7.14            | 57               |
| BM:CM 20:80 | 6.24       | 7.14            | 52               |

Initial pH was measured on one reactor per treatment after mixing; final pH is the mean of the replicate reactors. VS-reduction values are reported descriptively because replicate-level VS-removal documentation was not available in the source manuscript.

### 3.3. Methane production from co-digestion

All mixtures showed a small methane peak on day 2, a period of low production up to about day 8, and then a main production phase between days 10 and 30 (Fig. 4a). The 80:20, 60:40, 50:50 and 40:60 mixtures followed similar trajectories, with sharp peaks of up to  $\approx 360$  mL/d on days 18 and 26 that were common to several mixtures. Their concurrence across treatments suggests that measurement timing or accumulated gas may have contributed to these peaks; they should therefore not be interpreted as discrete biological events. The CM-rich 20:80 mixture started more slowly and produced methane steadily from day 30 onwards. Its curve contains a late increase on days 58-59, which is treated cautiously below.

After 62 days, four of the five mixtures (80:20, 50:50, 40:60 and 20:80) had mean measured

yields of 137-154 mL CH<sub>4</sub>/g VS added, compared with 109 and 133 mL/g VS added for BM and CM alone (Table 4; Fig. 6). The 60:40 treatment yielded 106 mL/g VS added from a single reactor. Replicate variability was substantial, with coefficients of variation of 23-32% for most treatments, and BMP differences among replicated treatments were not statistically significant (one-way ANOVA,  $F = 0.50$ ,  $p = 0.77$ ; all Tukey pairwise  $p \geq 0.76$ ). The reported VS reduction was higher in the mixtures (52-63%) than in the mono-digestion reactors (45-49%) (Table 3), but these values are interpreted descriptively. Thus, the data support faster kinetics more strongly than a treatment effect on ultimate methane yield.

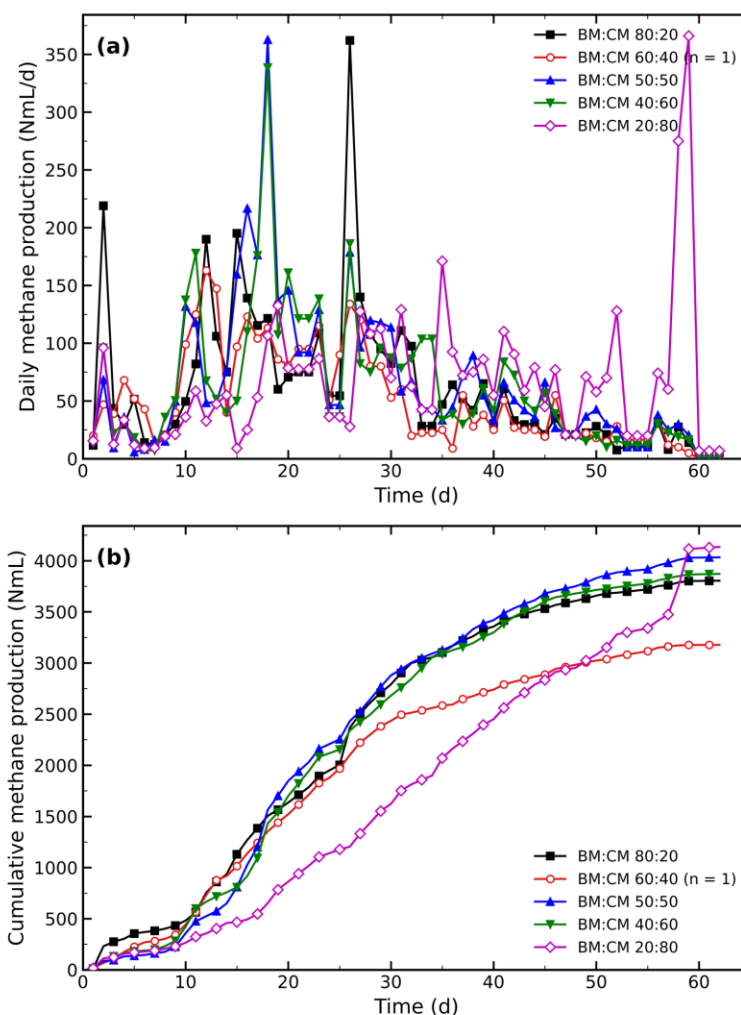


Fig. 4. (a) Daily and (b) cumulative methane production during co-digestion of buffalo manure and cow manure at BM:CM ratios (VS basis) of 80:20, 60:40, 50:50, 40:60 and 20:80 at 37 °C.

### 3.4. Kinetic analysis using the modified Gompertz model

The parameters of the modified Gompertz model (Section 2.6) are summarised in Table 4, and the fitted curves are compared with the measured data in Fig. 5. The model described the mean curves well, with  $R^2 = 0.987-0.998$  and  $RMSE = 1.9-5.4$  mL/g VS. For six of the seven treatments, the

predicted methane potential (P) was within  $-2.4\%$  to  $+8.2\%$  of the measured yield at day 62. The exception was 20:80, whose curve was still rising at day 62. Its fitted P (201 mL/g VS) exceeds the measured yield by 31% and should be regarded as an extrapolation rather than a directly comparable ultimate yield.

Table 4. Modified Gompertz model parameters for the mono- and co-digestion of buffalo manure (BM) and cow manure (CM).

| BM:CM      | Meas.<br>yield<br>(mL/g<br>VS) | P<br>(mL/g<br>VS)         | Rm (mL/g<br>VS/d) | $\lambda$ (d) | $t_i$ (d) | $T_{80}$<br>(d) | Diff.<br>(%) | $R^2$ | RMSE |
|------------|--------------------------------|---------------------------|-------------------|---------------|-----------|-----------------|--------------|-------|------|
| BM (100:0) | 109 ± 26                       | 106.6 ± 1.2               | 3.26 ± 0.10       | 5.2 ± 0.5     | 17.2      | 41              | -2.4         | 0.991 | 3.3  |
| CM (0:100) | 133 ± 43                       | 144.2 ± 1.3               | 3.99 ± 0.05       | 14.4 ± 0.2    | 27.7      | 44              | +8.2         | 0.998 | 1.9  |
| 80:20      | 137 ± 7                        | 141.5 ± 1.4               | 5.21 ± 0.16       | 7.3 ± 0.4     | 17.3      | 30              | +3.2         | 0.991 | 4.5  |
| 60:40      | 106                            | 106.2 ± 0.5               | 4.65 ± 0.09       | 7.1 ± 0.2     | 15.5      | 27              | +0.5         | 0.997 | 2.1  |
| 50:50      | 149 ± 44                       | 146.5 ± 0.9               | 6.49 ± 0.15       | 10.1 ± 0.3    | 18.4      | 32              | -1.4         | 0.996 | 3.4  |
| 40:60      | 140 ± 42                       | 141.9 ± 0.9               | 5.78 ± 0.12       | 9.4 ± 0.3     | 18.5      | 32              | +1.1         | 0.997 | 2.9  |
| 20:80      | 154 ± 35                       | 200.9 ± 13.1 <sup>a</sup> | 3.28 ± 0.08       | 14.8 ± 0.6    | 37.3      | 58              | +30.8        | 0.987 | 5.4  |

Meas. yield = measured blank-corrected cumulative methane at day 62 (mean ± SD of replicate reactors; 60:40, n = 1). P = methane production potential; Rm = maximum methane production rate;  $\lambda$  = lag phase;  $t_i$  = time of maximum production rate;  $T_{80}$  = time to reach 80% of the measured yield; Diff. =  $(P - \text{measured})/\text{measured} \times 100$ ; RMSE in mL/g VS. P, Rm and  $\lambda$  are given ± SE of the fit to the mean curve. <sup>a</sup> Curve had not reached a plateau; P is an extrapolation. Measured yields did not differ significantly among replicated treatments (ANOVA, p = 0.77; 60:40 excluded).

The lag phase ( $\lambda$ ) showed the clearest difference between the two manures. BM started producing methane after 5.2 ± 0.5 d, whereas CM required 14.4 ± 0.2 d. The longer lag of CM is consistent with its more acidic initial pH (6.04), which would prolong the transition from acid production to methanogenesis. Buffalo manure, by contrast, tends to restore neutral pH during digestion (Carotenuto et al., 2020). In the mixtures,  $\lambda$  lay between the values of the two manures and increased with the CM fraction: from 7.1-7.3 d at 60-80% BM, to 9.4-10.1 d at 40-50% BM and 14.8 d at 20% BM, where the mixture behaved essentially like CM.

The maximum methane production rate (Rm) showed the clearest response to co-digestion. BM

and CM alone reached 3.26 and 3.99 mL/g VS/d, respectively. The 80:20, 60:40, 50:50 and 40:60 mixtures reached 4.65-6.49 mL/g VS/d, all above the fitted rates of both manures. The highest fitted rate occurred at 50:50 and was about twice that of BM and 1.6 times that of CM. This pattern indicates accelerated methane-production kinetics in these mixtures; however, because the kinetic models were fitted to mean curves, the model-parameter SE does not represent between-reactor biological variability. The 20:80 mixture (3.28 mL/g VS/d) did not show the same acceleration. The faster kinetics shortened the technical digestion time. The 80:20 to 40:60 mixtures reached 80% of their final yield within 27-32 d, compared with 41 d for BM and 44 d for CM, a reduction of 9-17 d. The 20:80 mixture needed 58 d, partly because of the late readings discussed in Section 3.6. Batch kinetics cannot be translated directly into continuous operation, but a shorter  $T_{80}$  suggests that blends with 40-80% BM could be digested at shorter hydraulic retention times than either manure alone, which would reduce the required digester volume. This should be tested in semi-continuous reactors. The standard errors in Table 4 describe the fit to the mean curve only. They do not include the variability between replicate reactors, which was considerably larger (Table 4, measured yields).

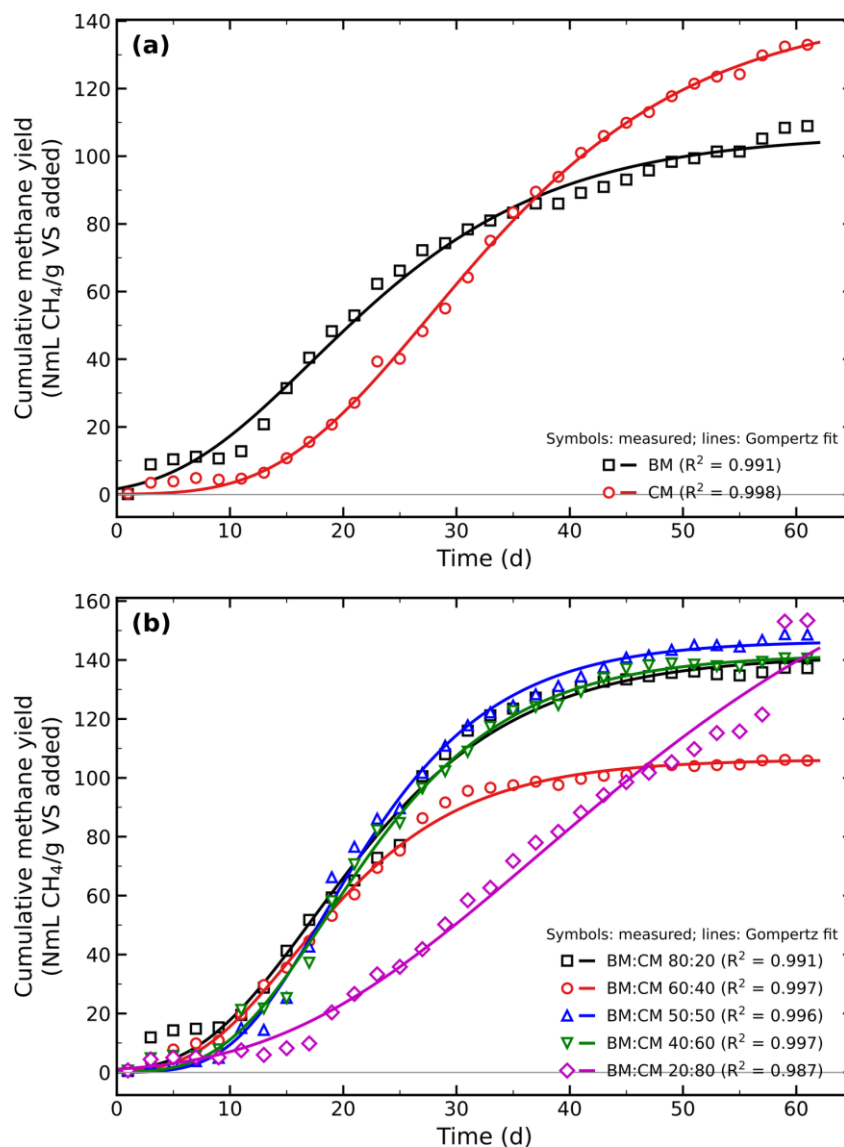


Fig. 5. Modified Gompertz model fits (lines) to the measured mean cumulative methane yields (symbols) for (a) mono-digestion and (b) co-digestion of buffalo manure (BM) and cow manure (CM). Fitted parameters are given in Table 4.

### 3.5. pH and VS reduction

The initial pH of the substrate-containing reactors (6.0-6.4) increased to 7.04-7.20 by the end of the assays (Table 3). All final values lay close to neutral pH, which is generally favourable for methanogenic activity (Carotenuto et al., 2020). Because VFAs and alkalinity were not measured, the pH recovery should not be interpreted as direct evidence that early VFA accumulation was

reversed or that buffering capacity was enhanced. The pH trend is nevertheless consistent with the reported tendency of buffalo-manure digestion to move from mildly acidic initial conditions towards neutrality (Carotenuto et al., 2020). In the blank, pH fell from 7.23 to 6.85. The reported VS reduction was highest for the 50:50 mixture (63%), followed by 60:40 (58%) and 40:60 (57%).

### 3.6. Substrate interactions

Table 5 and Fig. 6 compare the experimental yields of the mixtures with theoretical yields calculated from the mono-digestion results. On a VS-added basis, four of the five mixtures showed positive departures from the weighted theoretical yield ( $\alpha = 1.14$ -1.23), whereas the single 60:40 reactor gave  $\alpha = 0.89$ . The uncertainty of  $\alpha$  for the replicated mixtures ( $SE = 0.14$ -0.28) was comparable with the departures from 1, so these values cannot be distinguished statistically from additive behaviour. They should therefore be described as apparent positive deviations rather than demonstrated biological synergy.

On the reported VS-removed basis, the mixtures produced 182-295 mL CH<sub>4</sub>/g VS removed and  $\alpha$

values of 0.72-1.11. These values do not indicate a consistent increase in methane recovered per unit of VS removed. The reported VS reduction was 52-63% in the mixtures and 45-49% in mono-digestion, suggesting that greater overall solids conversion may have contributed to the higher apparent VS-added yields. However, replicate-level VS-removal documentation was not available, and C/N ratio, alkalinity, ammonia, VFAs and fibre composition were not measured. Consequently, proposed explanations involving buffering, nutrient balance, hydrolysis or acid consumption remain hypotheses for future targeted experiments.

**Table 5. Experimental and theoretical methane yields and synergy index ( $\alpha$ ) of the co-digestion mixtures on VS-added and VS-removed bases.**

| BM:CM | Exp. (VS added) | Theor. (VS added) | $\alpha$ (VS added) $\pm$ SE | Exp. (VS removed) | Theor. (VS removed) | $\alpha$ (VS removed) |
|-------|-----------------|-------------------|------------------------------|-------------------|---------------------|-----------------------|
| 80:20 | 137             | 114               | $1.20 \pm 0.14$              | 249               | 249                 | 1.00                  |
| 60:40 | 106             | 119               | 0.89 <sup>a</sup>            | 182               | 254                 | 0.72 <sup>a</sup>     |
| 50:50 | 149             | 121               | $1.23 \pm 0.26$              | 236               | 257                 | 0.92                  |
| 40:60 | 140             | 124               | $1.14 \pm 0.28$              | 246               | 260                 | 0.95                  |
| 20:80 | 154             | 128               | $1.20 \pm 0.27$              | 295               | 266                 | 1.11                  |

Yields in mL CH<sub>4</sub>/g VS. Theoretical yields from Eq. (5) using mono-digestion yields of 109 (BM) and 133 (CM) mL/g VS added, and 243 (BM) and 272 (CM) mL/g VS removed. <sup>a</sup> Single reactor; no SE.

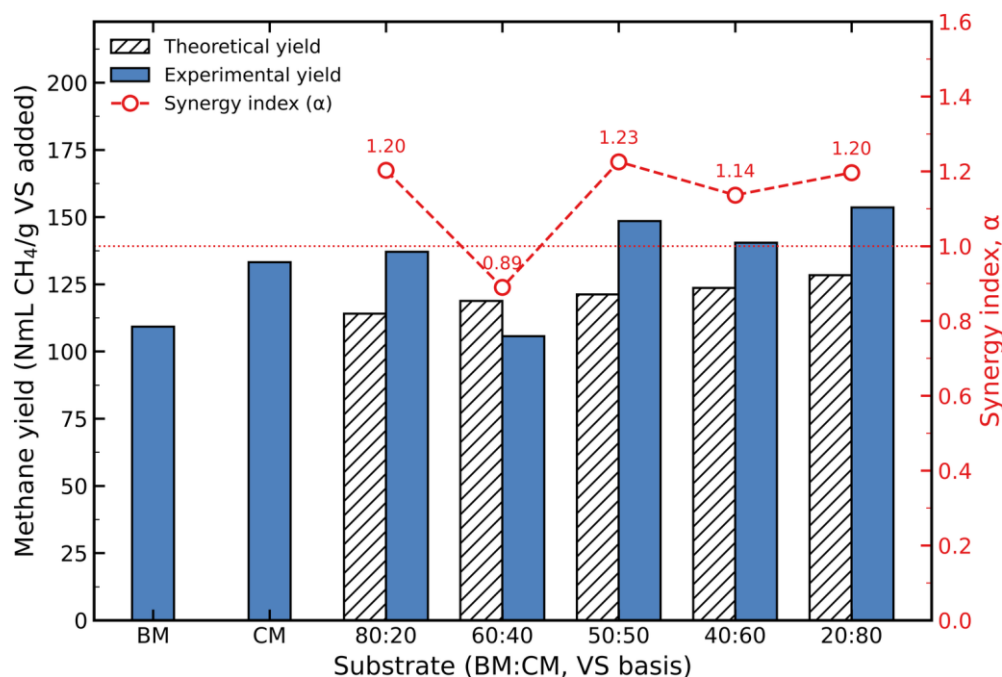


Fig. 6. Experimental and theoretical methane yields (VS-added basis) of mono- and co-digestion, and the synergy index ( $\alpha$ ) of each mixture. The dotted line marks  $\alpha = 1$ .

The 20:80 result requires particular caution. One of its two reactors recorded 520 and 660 mL of methane on days 58 and 59, compared with less than 90 mL/d in the preceding week and 8 mL/d afterwards. These two readings alone add about 59 mL/g VS to that reactor's yield. A sensitivity calculation excluding these two values gives an approximate treatment yield of 124 mL/g VS added ( $\alpha \approx 0.97$ ), rather than the reported 154 mL/g VS ( $\alpha = 1.20$ ). Because the source record does not establish whether the late values represent accumulated gas or measurement/recording error, the original observations are retained in the primary dataset but are not used as evidence of synergy. The sensitivity result demonstrates that the interpretation of this treatment is highly dependent on those two observations.

Table 6 compares these results with other studies on manure mono- and co-digestion. Positive co-digestion effects have been reported for cow and sheep manure, which gave a best batch yield of 210 mL/g VS added at a CM:SM ratio of 1:3 (Li et al., 2020). In other buffalo- and cattle-dung blends, favourable ratios have typically contained 40-75% dung (Basumatary et al., 2024; Kandhro et al., 2025; Korai et al., 2024). In the present study, the strongest and most consistent response was kinetic rather than an increase in ultimate methane yield. Mixtures containing 40-80% BM reached 80% of their measured final yield sooner than either manure alone, while final BMP differences were not statistically significant. These results support further evaluation of flexible BM-CM blending in semi-continuous digestion rather than selection of a single optimum ratio from the present batch dataset.

**Table 6. Comparison with studies on manure mono- and co-digestion.**

| Study                       | Substrates                               | Conditions                         | Best ratio             | Key result                                                              |
|-----------------------------|------------------------------------------|------------------------------------|------------------------|-------------------------------------------------------------------------|
| This study                  | Buffalo + cow manure                     | Batch, 37 °C, 62 d                 | No significant optimum | 137-154 mL CH <sub>4</sub> /g VS added; $\alpha = 1.14-1.23$ (VS added) |
| (Li et al., 2020)           | Cow + sheep manure                       | Batch, 37 °C                       | CM:SM (VS) 1:3         | 210 mL CH <sub>4</sub> /g VS added; synergy reported                    |
| (Jo et al., 2024)           | Slaughterhouse waste + cattle/pig manure | Batch, BMP                         | CM:SHW 1:1             | SI = 1.18 (CM), 1.10 (PM); shorter lag                                  |
| (Kandhro et al., 2025)      | Fruit-vegetable waste + buffalo dung     | Batch                              | FVW:BD 40:60 (VS)      | 258 mL CH <sub>4</sub> /g VS; Gompertz fitted                           |
| (Korai et al., 2024)        | Buffalo dung + petroleum sludge          | Batch                              | 1.5:0.5                | 326 mL CH <sub>4</sub> /g VS                                            |
| (Basumatary et al., 2024)   | Cattle dung + cooked kitchen waste       | Batch, 35 °C                       | CD:CKW 40:60           | ≈250 mL CH <sub>4</sub> /g VS (with biochar)                            |
| (Kafle & Chen, 2016)        | Dairy (mono) manure                      | Batch, mesophilic                  |                        | 204 mL CH <sub>4</sub> /g VS; 58.6% VS removal                          |
| (Yu et al., 2024)           | Cabbage waste + cattle manure            | Batch and continuous               | 3:1                    | 440 mL CH <sub>4</sub> /g VS; synergy (CPI > 1)                         |
| (Kandhro et al., 2025)      | Buffalo (mono) dung                      | Full-scale dome digester, 26-36 °C |                        | 0.23-0.38 m <sup>3</sup> biogas/kg VS added                             |
| (Iqbal Jarwar et al., n.d.) | Poultry manure + buffalo dung            | Batch, 37 °C, 65 d                 | PM:BD 1:3              | 561 mL biogas/g VS                                                      |
| (Zhou et al., 2023)         | Buffalo manure + excess sludge           | Batch, 35/55 °C                    | BM:ES 1:1.5            | Yield +88-153% vs sludge alone (mesophilic)                             |

### 3.7. Limitations

Several limitations should be considered when using these results. (i) The substrates were sampled once, from one farm, and digested with a single inoculum, so seasonal and farm-to-farm variation was not captured. (ii) Methane was measured manually by syringe after alkaline CO<sub>2</sub> absorption, a method prone to operator error and to temperature disturbance when bottles are removed for measurement (Filer et al., 2019). Isolated daily readings, such as the days 58-59 values for 20:80, therefore need independent verification. (iii) C/N ratio, fibre composition,

alkalinity, VFAs and ammonia were not measured, so the mechanisms proposed in Section 3.6 remain hypotheses. (iv) Batch BMP tests indicate methane potential and relative kinetics but do not predict the stability of continuously fed digesters at practical retention times and loading rates.

### 4. Conclusions

This study assessed the mesophilic batch co-digestion of buffalo and cow manure generated together in Karachi's cattle colonies. Buffalo manure exhibited a fitted lag phase about 9 days shorter than cow manure, whereas cow manure

had the higher mean final methane yield. The clearest effect of co-digestion was accelerated methane-production kinetics: mixtures containing 40-80% BM reached higher fitted maximum production rates, with the highest Rm observed at 50:50, and reached 80% of their measured final yield 9-17 days sooner than the mono-digestion treatments. Although four mixtures showed apparent positive deviations of 14-23% from weighted theoretical yields, BMP differences among replicated treatments were not statistically significant and the uncertainty of the interaction index did not establish biological synergy. The 20:80 treatment was additionally sensitive to two anomalously high late measurements. The batch data therefore indicate that BM and CM can potentially be co-digested across a broad range of ratios without a detectable methane-yield penalty, while several mixtures show faster production kinetics. Better-replicated and semi-continuous experiments, with monitoring of C/N ratio, alkalinity, VFAs, ammonia and replicate-level VS removal, are required before defining operational feed ratios or full-scale retention times.

## CRedit authorship contribution statement

**Abdul Fatah Soomro:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Irfan Ahmed Abbasi:** Methodology, Formal analysis, Writing review & editing. **Aurangzeb Junejo:** Investigation, Data curation, Formal analysis, Writing original draft. **Khuda Buksh Jokhio:** Investigation, Data curation, Writing original draft. **Siraj Ahmed:** Investigation, Data curation, Writing original draft. **Mansoor Ahmed:** Investigation, Data curation, Formal analysis, Writing original draft. **Asadullah Memon:** Investigation, Data curation, Formal analysis, Writing original draft.

## 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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