Comparative study between mono-digestion and co-digestion of Terrestrial weed (Lantana camara)

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Abstract: - Lantana camara is widely recognised as one of the twenty most invasive terrestrial weeds due to its rapid growth and high reproductive capacity. It can spread quickly and dominate forest ecosystems within a short period. Control and eradication of this species are difficult because of its strong regenerative ability. However, its abundant availability makes it a potential lignocellulosic feedstock for renewable energy generation through anaerobic digestion. In mono-digestion, methane production occurs but at relatively lower yields due to the accumulation of inhibitory compounds. Therefore, co-digestion is often employed to improve nutrient balance and reduce the impact of toxic inhibitors. In biochemical methane potential (BMP) assays, an F/M ratio of 1.5 was identified as optimal for the anaerobic digestion of Lantana camara, producing 191 mL CH_4 g^{-1} VS on day 26. In co-digestion BMP assays, the same ratio resulted in a higher methane yield of 211 mL CH_4 g^{-1} VS by day 14, indicating improved degradation kinetics. Additionally, the hydrolysis phase was shorter in co-digestion compared with mono-digestion. Scale-up experiments were conducted in a 20 L batch reactor with a 15 L working volume to validate the optimal F/M ratios obtained from BMP assays. The results demonstrated improved biogas production performance. Batch reactor studies further suggested an optimum hydraulic retention time (HRT) of 13 days for achieving peak methane production during anaerobic co-digestion of Lantana camara. Subsequently, a continuous anaerobic digester was operated for 60 days using the optimal F/M ratio determined from the BMP experiments.

Key-Words: - Lantana camara; anaerobic digestion; biochemical methane potential; co-digestion; F/M ratio.

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1 Introduction

India is a rapidly growing nation. As of mid-April 2026, its population stands at approximately 1.474 billion, per Worldometer's UN-based live counter. Rapid population growth and industrial expansion have significantly increased the country's energy demand [2]. At present, conventional non-renewable energy sources such as coal, oil, and natural gas play a dominant role in meeting national energy requirements. However, India remains highly dependent on imported petroleum resources. Reports indicate that a substantial portion of the national energy demand has historically been met through oil imports, with diesel consumption accounting for nearly 43.2% of the total petroleum usage during 2000–2001 [3]. Global studies have also suggested that fossil fuel reserves are finite, with projections indicating that world oil reserves could be largely depleted by the mid-21st century. Moreover, the excessive use of fossil fuels contributes significantly to environmental pollution and climate change, which are associated with severe public health impacts. The World Health Organisation has linked a considerable number of premature deaths annually to environmental and climate-related factors [1]. Consequently, the development and utilisation of renewable energy resources have become essential for sustainable energy supply and environmental protection. Biofuels derived from renewable biomass, including biogas, biodiesel, bio-oil, producer gas, and bio-methane, have attracted considerable research interest as potential alternatives to fossil fuels [4]

Weeds are generally defined as undesirable plants that grow in areas where they are not wanted. Despite their negative impact on agricultural productivity, weeds can serve as a potential biomass resource for renewable energy generation. Several terrestrial weed species present significant ecological and economic challenges in India and other parts of the world, including Parthenium hysterophorus, Lantana camara, Ageratum conyzoides, Galinsoga parviflora, Saccharum spontaneum, and Argemone mexicana. Among these, Lantana camara, commonly known as wild sage or red sage, is considered one of the most invasive weed species globally [6]. The species name L. camara is believed to originate from the West Indian informal name used for this plant [7], [8]. This species exhibits high regenerative ability and continuous reproductive potential, which facilitates its rapid spread across invaded ecosystems. Due to its dense and bushy growth pattern, L. camara often forms thick vegetation layers in forest and plantation areas, suppressing native plant species and increasing wildfire risks in many forested regions of India [1], [45].

The conversion of such invasive biomass into renewable energy through anaerobic digestion represents an environmentally sustainable management strategy. Anaerobic digestion is a widely established biological process for biogas production, enabling the conversion of various organic wastes into methane-rich biogas. This technology has gained global attention due to its economic viability and environmental benefits [4]. Although several studies have investigated pretreatment methods and co-digestion strategies for improving biogas production from lignocellulosic biomass [10], a detailed comparative biochemical methane potential (BMP) assessment between mono-digestion and co-digestion of L. camara remains limited

2 Materials and methods

2.1 Substrate and inoculum

Lantana camara was collected from areas surrounding the Indian Institute of Technology Guwahati (IIT Guwahati), Assam, India. Cow dung, used as the inoculum in the anaerobic digestion experiments, was obtained from a farm located in Amingaon village near the IIT Guwahati campus. The collected *L. camara* biomass was first chopped into smaller pieces and then ground using a grinder to increase the surface area and enhance biodegradability during digestion. For the co-digestion experiments, food waste was collected from various hostel mess facilities within the IIT Guwahati campus. The food waste was manually segregated to maintain uniform composition before use. The initial physicochemical characteristics of *L. camara*, food waste, and cow dung are presented in Table 1.

Table 1. Initial characterisation of Lantana camara food waste and cow dung

Parameters	*Lantana camara*	Cow dung	Food waste
pH	6.73	7.15	4.2
MC (%)	76.3	81	74
sCOD (mg/L)	6552	2343	2000
VFA (mg/L)	390	620	1250
VS (%)	18.08	15.69	21

2.1 Mono digestion of terrestrial weeds using cow dung as inoculum

Anaerobic digestion (AD) experiments were performed at different substrate-to-inoculum mixing ratios to determine the optimal food-to-microorganism (F/M) ratio for methane production [11]. Biochemical methane potential (BMP) assays were conducted in 1 L glass reactor bottles using the liquid displacement method to measure biogas generation [14]. The AD of *Lantana camara* was carried out using fresh cow dung as the inoculum, as illustrated in Figure 1a. Experiments were performed at F/M ratios of 1.0, 1.5, 2.0, and 2.5, with each condition tested in triplicate to ensure reproducibility (Table 2). Control reactors containing only *L. camara* and only cow dung were also included in the experimental setup.

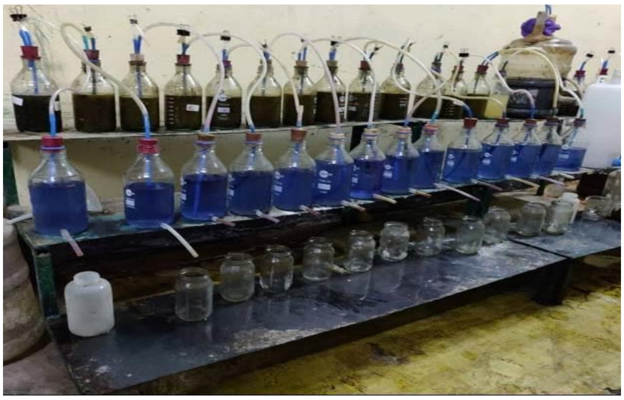

Fig. 1: a. Biochemical Methane Potential (BMP) Assay

Table 2. Quantity of substrate (Lantana camara) and Inoculum (cow dung) used for different F/M ratios

F/M ratio	*Lantana camara* (g)	Cow dung (g)
Control 1	----	50
Control 2	50	----
1.0	43.39	50
1.5	65.08	50
2.0	86.78	50
2.5	108.48	50

2.2 Anaerobic co-digestion of terrestrial weeds using food waste as co-substrate and cow dung as inoculum

The biochemical methane potential (BMP) assay for the anaerobic co-digestion of *Lantana camara* and food waste was conducted using cow dung as the inoculum. Experiments were performed at food-to-microorganism (F/M) ratios of 1.0, 1.5, 2.0, and 2.5, with each condition tested in triplicate (Table 3). Control reactors containing only *L. camara* and only cow dung were also included for comparison.

Table 3. Quantity of Lantana camara, food waste and cow dung used for various mixing ratios based on VS

Mixing ratio	*Lantana camara* (g)	Cow dung (g)	Food waste (g)
Control 1	----	50	----
Control 2	50	----	----
1.0	21.69	50	18.68
1.5	32.54	50	28.02
2.0	43.39	50	37.36
2.5	54.24	50	46.70

2.3 Initial characterization

The initial characterisation was performed to determine the physicochemical properties of the substrate and inoculum. Parameters including moisture content (MC), total solids (TS), volatile solids (VS), volatile fatty acids (VFA), and soluble chemical oxygen demand (sCOD) were analysed according to standard procedures described in APHA (2005).

2.4 Anaerobic batch study to optimise F/M ratio

Biochemical methane potential (BMP) assays were conducted in 1 L glass reagent bottles containing the substrate and inoculum [17]. The quantities of substrate and inoculum were determined based on volatile solids (VS) content. Food-to-microorganism (F/M) ratios of 1.0, 1.5, 2.0, and 2.5 were evaluated along with two control setups containing only inoculum and only substrate. Macro- and micronutrients, including phosphate buffer, ferric chloride, calcium chloride, magnesium sulfate, and cobalt nitrate, were added to each reactor bottle to support microbial activity [15]. The working volume in each bottle was adjusted to 700 mL using distilled water.

In this study, *Lantana camara* was used as the substrate, while fresh cow dung served as the inoculum. All experiments were performed in triplicate for each F/M ratio. To maintain anaerobic conditions, nitrogen gas was purged through the reactors for 3 min before sealing. The bottles were then tightly closed with rubber stoppers and connected to aspirator bottles containing 1.5 N NaOH solution mixed with thymol blue alkali indicator (Figure. 1a and 1b).

Biogas production was measured daily using the liquid displacement method. Methane produced in the reactor passed through the NaOH solution under pressure, where NaOH absorbed carbon dioxide to form sodium carbonate. The volume of methane produced was determined from the displacement of the NaOH solution, and the displaced liquid was measured using a graduated measuring cylinder [26].

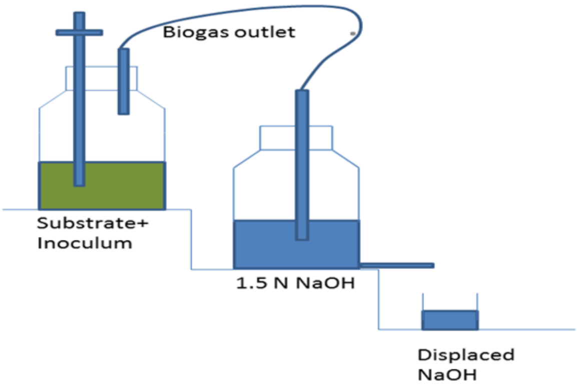

Fig. 2: b. presents a detailed diagrammatic representation of the biochemical methane potential (BMP) assay setup, clearly depicting all essential components and their interconnections throughout the experimental process

2.5 Continuous digester

A two-stage steel biogas digester was employed as a continuous reactor for the anaerobic digestion experiments. The cylindrical reactor had a total volume of 20 L and was equipped with an internal agitator assembly consisting of four blades to ensure proper mixing and enhance digestion efficiency. The schematic representation of the continuous reactor is shown in Figure. 1c.

The digester was fitted with separate inlet and outlet ports for substrate feeding and digestate removal. In addition, gas outlet and sampling ports were provided for biogas collection and monitoring. The gas outlet was connected to an aspirator bottle containing NaOH solution to absorb carbon dioxide. Methane production was determined using the liquid displacement method based on the volume of displaced NaOH solution. Table 4. Showed the amount of substrate fed for mono digestion and co-digestion.

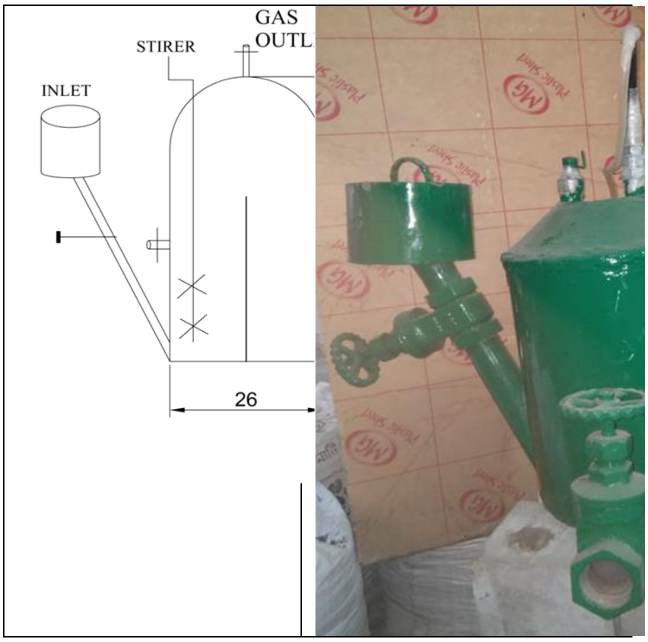

Fig. 1: c. Figure X illustrates both the detailed schematic design of the continuous anaerobic reactor system and its complete experimental configuration, highlighting key components such as the feed inlet, mixing mechanism, temperature control unit, gas collection system and effluent outlet , providing a comprehensive visual representation of the operational workflow for sustained biogas production from Lantana camara co-digestion

Table 4. Daily substrate feeding amounts for mono digestion and codigestion (Lantana camara + food waste) in a semi-continuous digester

ORL (KgCOD/m3 D	Untreated	Co-Digestion
Lantana camara	4.1	6.9

2.6 Kinetic Modelling

Cumulative methane production curves were fitted using the modified Gompertz equation to evaluate methane production potential (P), maximum production rate (R_m), and lag phase (λ):

$M(t)=P\exp\{-\exp[PRm$
$(\lambda-t)+1]\}$……………equation 1

Here, $M(t)$ is cumulative CH_4 production (mL g^{-1} VS), t is digestion time (days), P is biogas potential, R_m is maximum production rate, and λ is lag phase duration.

Non-linear regression was performed using OriginPro 2025. Model goodness-of-fit was assessed via R^2 (>0.98) and RMSE [44].

3 Result and discussion

3.1 Biogas yield during mono digestion and co-digestion

The biochemical methane potential (BMP) assay was conducted at different F/M ratios (1.0, 1.5, 2.0, and 2.5) for a duration of 49 days, during which the ambient temperature ranged between 30 and 40 °C. Among the tested conditions, an F/M ratio of 1.5 exhibited the highest methane yield, followed by ratios of 2.0 and 2.5, as illustrated in the figure. 2a. The maximum methane yield of 191 mL CH_4 g^{-1} VS was observed on the 26th day at an F/M ratio of 1.5. Methane generation is directly associated with the extent of substrate biodegradation during anaerobic digestion [21]. Typically, the hydrolysis stage is relatively slow, particularly for lignocellulosic substrates, which delays methane production [25]. However, in the case of the F/M ratio of 1.5, the hydrolysis phase was shorter compared with other tested ratios, indicating enhanced degradation efficiency [16]. Previous studies have also reported that methane production depends strongly on the biodegradation rate of the substrate [10].

The cumulative methane production was also highest at an F/M ratio of 1.5, reaching 4152 mL, followed by ratios of 2.0 and 2.5 (Figure 3a). Methane production increased as the F/M ratio increased from 1.0 to 1.5, but decreased when the ratio was further increased to 2.0 and 2.5. Since lignocellulosic biomass constitutes the primary substrate in Lantana camara, the initial methane production required a longer adaptation period [19]. At an F/M ratio of 1.0, the substrate concentration was relatively low compared with the inoculum, resulting in reduced methane yield. Conversely, at higher F/M ratios (≥2.0), excessive substrate loading may have led to the accumulation of inhibitory compounds such as ammonia and other nitrogenous compounds, which can suppress microbial activity and reduce methane production [23]. Nevertheless, methane yields obtained at all tested F/M ratios were higher than those observed in the control reactor containing only cow dung (50 g) [22].

To evaluate the effect of co-digestion, additional experiments were conducted using Lantana camara and food waste with cow dung as the inoculum [20]. Food waste is generally more biodegradable and contains lower lignocellulosic content than plant biomass [6]. The results indicated that an F/M ratio of 1.5 again produced the highest methane yield of 211 mL CH_4 g^{-1} VS on the 14th day (Figure. 2b). Compared with mono-digestion, the hydrolysis phase during co-digestion was significantly shorter, resulting in earlier methane production [18]. In mono-digestion, the maximum methane yield was observed only after 26 days. The cumulative methane production during co-digestion reached a maximum of 5026 mL at the F/M ratio of 1.5 (Figure 3a and 3b). The reduced hydrolysis period during co-digestion can be attributed to the readily degradable nature of food waste and its low lignin content, which facilitates microbial degradation and reduces the lag phase [24]. The cumulative methane production followed the order: 1.5 > 2.0 > 1.0 > 2.5.

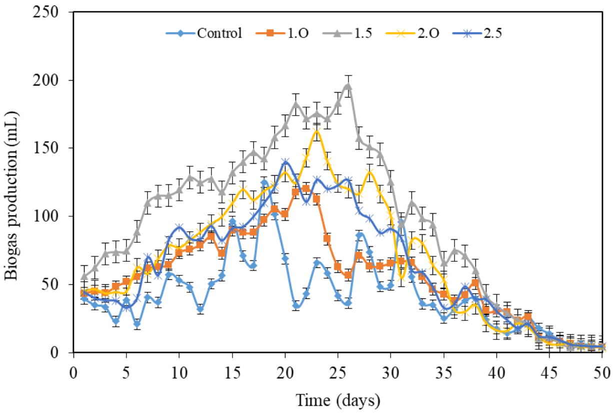

Fig. 2: a illustrates the daily methane yield trends observed during anaerobic digestion (AD) of Lantana camara biomass tested at multiple food-to-microorganism (F/M) ratios, highlighting peak production periods and comparative performance differences among the tested conditions over the 50-day experimental duration

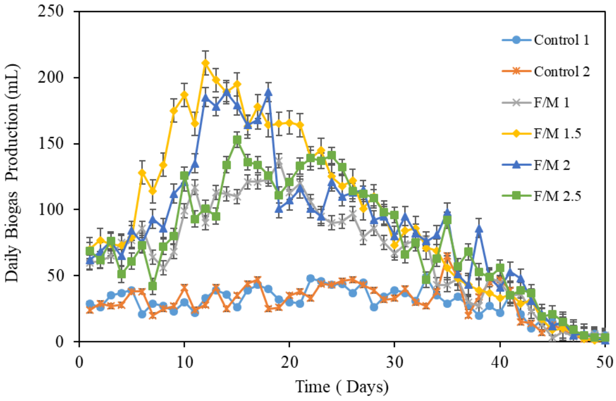

Fig. 2: b displays the daily methane production patterns achieved through co-digestion during anaerobic digestion (AD) of Lantana camara biomass tested at various food-to-microorganism (F/M) ratios, revealing accelerated peak yields and enhanced cumulative output compared to mono-digestion, with optimal performance evident at the F/M ratio of 1.5 over the experimental period

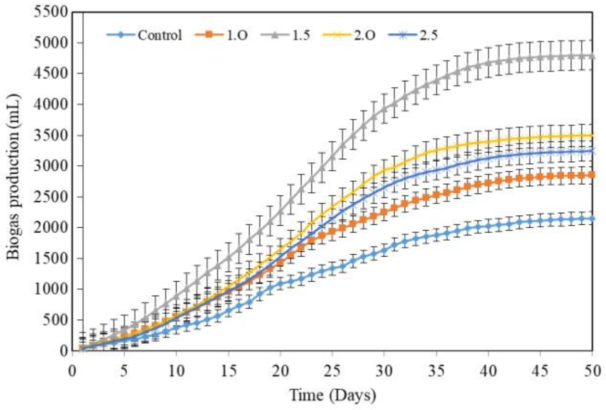

Fig. 3: a. Presents the cumulative methane production curves obtained from anaerobic digestion (AD) of Lantana camara biomass evaluated at various food-to-microorganism (F/M) ratios

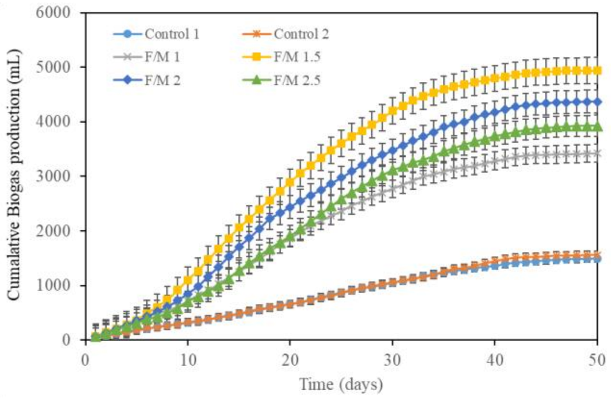

Fig. 3: b. Depicts the cumulative methane production profiles achieved through co-digestion of Lantana camara biomass tested at various mixing ratios

3.2 Volatile solids (VS) reduction.

The reduction of volatile solids (VS) reflects the extent of organic matter degradation during anaerobic digestion and is largely influenced by microbial activity. In the mono-digestion of Lantana camara, a gradual decrease in VS content was observed over time (Figure. 4a). However, the observed decline remained relatively moderate, attributable to the inherent lignocellulosic composition of the substrate, which provides structural recalcitrance and sustained slow-release of fermentable organics during extended digestion [27]. Among the tested conditions, the F/M ratio of 1.5 exhibited the highest VS reduction of 35.5%, followed by F/M ratios of 2.0 and 2.5. Higher VS reduction at the F/M ratio of 1.5 corresponded with increased methane and cumulative methane production. In contrast, control 2 showed the lowest VS reduction, while all experimental F/M ratios demonstrated higher VS removal compared with the control. Previous studies have also reported that greater VS reduction generally leads to enhanced biogas production [17]. In co-digestion experiments, varying mixing ratios resulted in different levels of volatile solids (VS) reduction, with optimal ratios demonstrating enhanced degradation efficiency due to balanced nutrient profiles and dilution of inhibitory compounds [29]. The mixing ratio of 1.5 again showed the highest VS reduction of 48.5% (Figure. 4b). A decreasing trend in VS content was observed throughout the digestion period. The higher VS removal during co-digestion can be attributed to improved microbial activity and the presence of easily degradable organic matter in food waste [28]. Overall, VS reduction in co-digestion was approximately 13% higher than that observed in mono-digestion (Figure 4a and Figure 4b). The VS reduction followed the order: 1.5 > 2.0 > 2.5 > 1.0 > control 1 > control 2, with all experimental conditions showing greater VS removal than control.

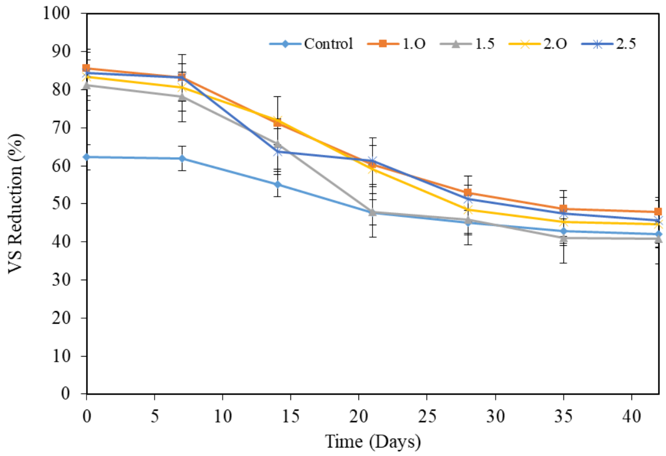

Fig. 4: a. illustrates the volatile solids (VS) reduction percentages achieved during anaerobic digestion (AD) of Lantana camara biomass across various food-to-microorganism (F/M) ratios

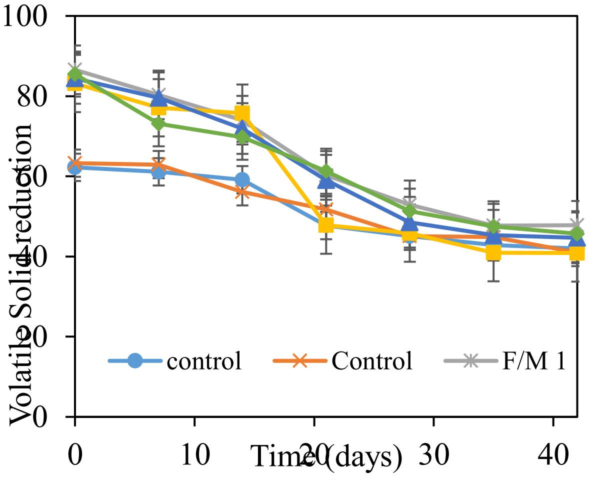

Fig. 4: b. illustrates the volatile solids (VS) reduction percentages achieved during co-digestion of Lantana camara biomass across various mixing ratios

2.3.3 Soluble oxygen demand (sCOD)

As shown in the figure. 5a, the soluble chemical oxygen demand (sCOD) increased from day 0 to day 28 for all tested F/M ratios and subsequently declined after the 28th day. The increase in sCOD indicates the hydrolysis of complex organic matter into soluble compounds, which are more readily available for microbial degradation [16]. The rise in sCOD during the initial digestion phase suggests active hydrolysis and solubilization of organic substrates. However, the decline observed after day 28 may be associated with the accumulation of inhibitory compounds generated during microbial metabolism, such as ammonia and sulfite, which can hinder further degradation of organic matter. Sulfite ions, in particular, are known to be toxic to anaerobic microorganisms and may suppress microbial activity [22].

Among the tested conditions, the highest sCOD value of 8192 mg L^{-1} was observed at an F/M ratio of 1.5, indicating enhanced substrate solubilization. At the lower F/M ratio of 1.0, the substrate concentration was relatively low compared with the inoculum, which limited the availability of degradable organic matter. In contrast, higher F/M ratios (>1.5) may have resulted in excessive substrate loading, which could negatively affect microbial activity and reduce degradation efficiency [12]. Therefore, an F/M ratio of 1.5 was considered optimal for effective digestion. Previous studies have also reported that increased sCOD levels are generally associated with higher volatile fatty acid (VFA) production and methane generation [7].

In the co-digestion experiments, sCOD exhibited a similar trend but reached its maximum earlier compared with mono-digestion. As shown in the figure. 5b, sCOD increased until the 14th day and decreased thereafter. The highest sCOD value of 9032 mg L^{-1} was observed during the second week of digestion at an F/M ratio of 1.5. The subsequent decrease in sCOD may be attributed to the accumulation of inhibitory compounds in the reactor system [30]. The sCOD values across the different mixing ratios followed the order: 1.5 > 2.0 > 2.5 > 1.0 > control 1 > control 2. The higher sCOD levels during co-digestion indicate the presence of more readily biodegradable organic matter, which enhances substrate conversion and biogas production [18].

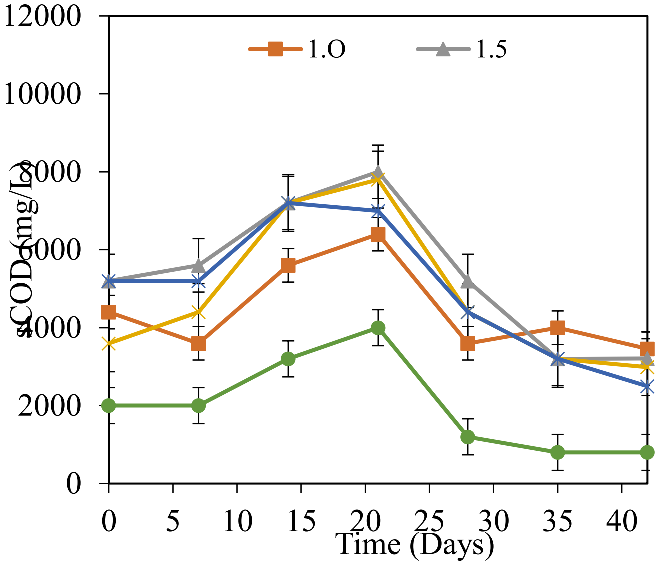

Fig. 5: a. Illustrates the variations in soluble chemical oxygen demand (sCOD) during anaerobic digestion (AD) of Lantana camara biomass across various food-to-microorganism (F/M) ratios

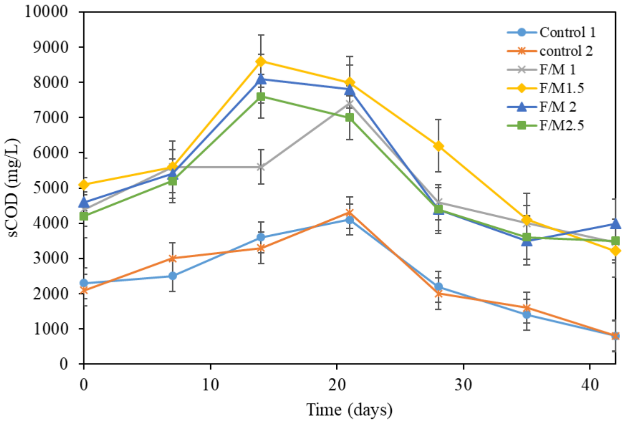

Fig. 5: b. illustrates the variations in soluble chemical oxygen demand (sCOD) observed during co-digestion of Lantana camara biomass across various mixing ratios

3.4 Volatile fatty acids (VFA)

Volatile fatty acids (VFAs) serve as key intermediate products generated during anaerobic digestion, formed through the hydrolysis of complex organic molecules into simpler soluble compounds during the acidogenesis phase [30]. During the acidogenesis stage, carbohydrates and other soluble substrates are converted into VFAs, which are subsequently transformed into acetic acid and finally into methane and carbon dioxide during methanogenesis [20]. As shown in Figure. 6a, VFA concentration increased until the 21st day due to the activity of acidogenic microorganisms. After the 21st day, VFA levels gradually declined, indicating the onset of the methanogenic phase, where VFAs were consumed by methanogens for methane production. The maximum VFA concentration was observed at an F/M ratio of 1.5 (1567 mg L^{-1}), followed by the F/M ratio of 2.0.

Variations in VFA concentration can significantly influence the pH of the anaerobic reactor, which in turn affects microbial activity. Maintaining an appropriate pH range is essential for stable methanogenic activity. High VFA levels reduce pH, creating toxic conditions that stress methanogens, especially in low-buffer systems where pH serves as a key imbalance indicator. Conversely, controlled pH (e.g., around 7.0) maximises VFA production during acidification while supporting downstream methanogenesis [41]. A decrease in pH below 6.0–6.5 may inhibit bacterial growth and reduce methane production [8]. Previous studies have reported that more than 75% methane production can be achieved when the pH remains above 5.0 [9], while methanogenic microorganisms generally perform optimally within a pH range of 6.6–7.6 [12]. Methanogens require pH 6.8–8.0 for peak activity, with drops below 6.2 sharply reducing methane yields due to inhibited VFA conversion. Some hydrogenotrophic strains tolerate pH 8.5–9.0 under thermophilic conditions, showing higher methane rates than at 7.0–7.5 [42]. Higher VFA production is often associated with increased methane generation due to the availability of readily degradable intermediates [13]. In the co-digestion experiments, a similar trend was observed; however, VFA accumulation occurred earlier compared with mono-digestion due to the presence of readily degradable food waste [31]. As shown in the figure. 6b, VFA concentration increased up to the 14th day and declined thereafter, indicating the transition from acidogenesis to methanogenesis [21]. The highest VFA concentration during co-digestion was 2692 mg L^{-1} on the 14th day at a mixing ratio of 1.5. The order of VFA concentration across different mixing ratios was 1.5 > 2.0 > 2.5 > 1.0 > control 1 > control 2, which is consistent with the observed trends in methane production.

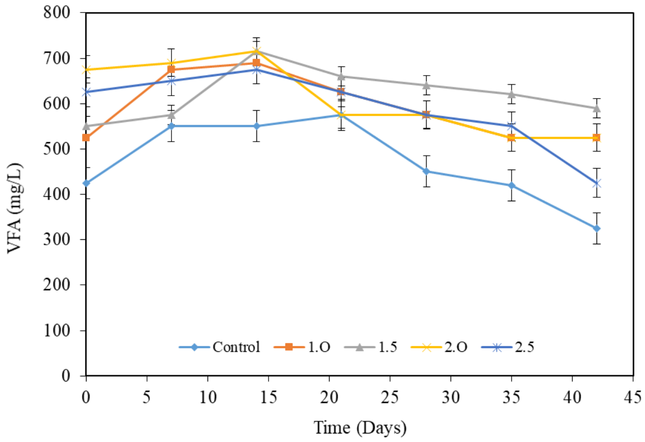

Fig. 6: a. illustrates the volatile fatty acid (VFA) concentration variations observed during anaerobic digestion (AD) of Lantana camara biomass across various food-to-microorganism (F/M) ratios,

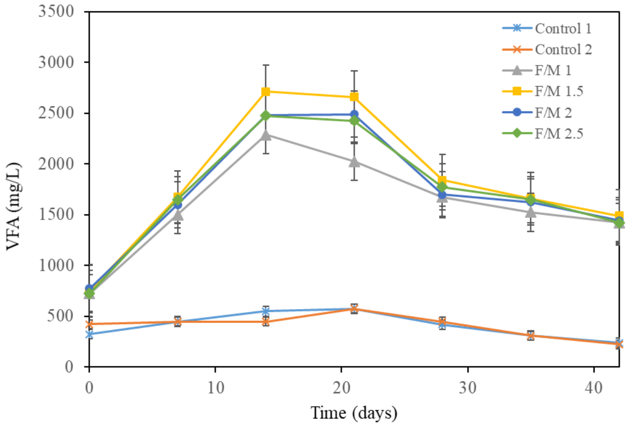

Fig. 6: b. Illustrates the volatile fatty acid (VFA) concentration variations observed during co-digestion of Lantana camara biomass across various mixing ratios

3.5 Batch Study

Following the 1 L BMP experiments, scale-up studies were conducted using a 20 L batch reactor with a working volume of 14 L at the optimal F/M ratio of 1.5. During the mono-digestion experiments, the highest methane yield was observed on the 25th day, reaching 3819 mL CH_4 g^{-1} VS. The cumulative methane production recorded in the 20 L batch reactor was 60,757 mL, as shown in Figure 7a and 7b.

Similarly, co-digestion experiments were performed in the 20 L batch reactor using the same optimal F/M ratio of 1.5. In this case, the maximum methane yield was achieved earlier, on the 13th day, with a value of 4374 mL CH_4 g^{-1} VS. The cumulative methane production reached 83,955 mL, as illustrated in the figure. 8a and 8b. The improved methane production and shorter digestion time during co-digestion can be attributed to the presence of easily degradable organic matter in food waste, which enhanced microbial activity and accelerated substrate degradation [13], [33], [34].

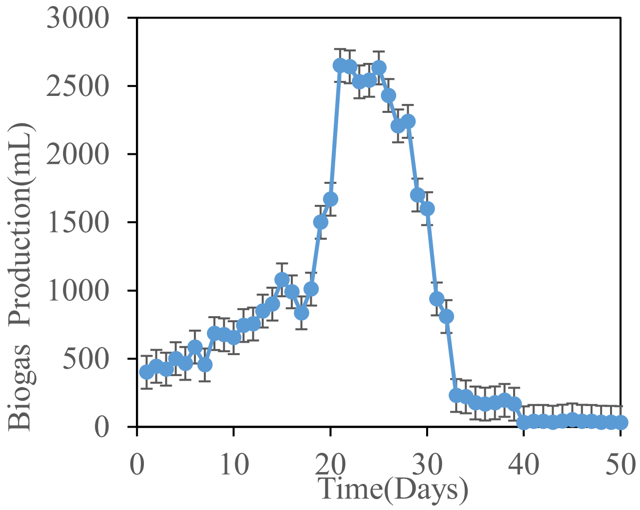

Fig. 7: a. Presents the daily methane production profile achieved in the 20 L batch reactor (15 L working volume) during anaerobic digestion of Lantana camara biomass at the optimal F/M ratio of 1.5

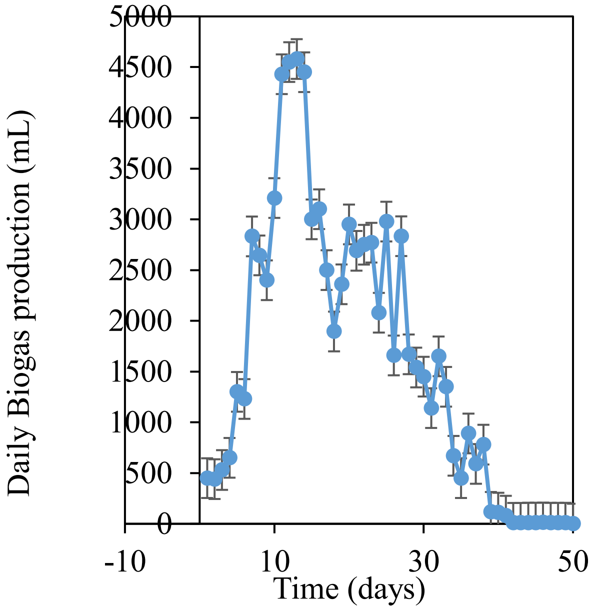

Fig. 7: b. Presents the daily methane production profile achieved in the 20 L batch reactor (15 L working volume) during co-digestion of Lantana camara with food waste at the optimal mixing ratio of 1.5

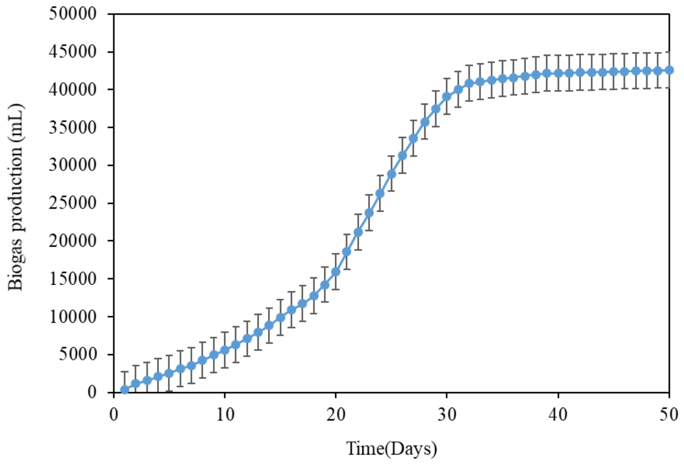

Fig. 8: a. Depicts cumulative methane production from anaerobic digestion of Lantana camara in a 20 L batch reactor at an optimal F/M ratio of 1.5, using cow dung as inoculum

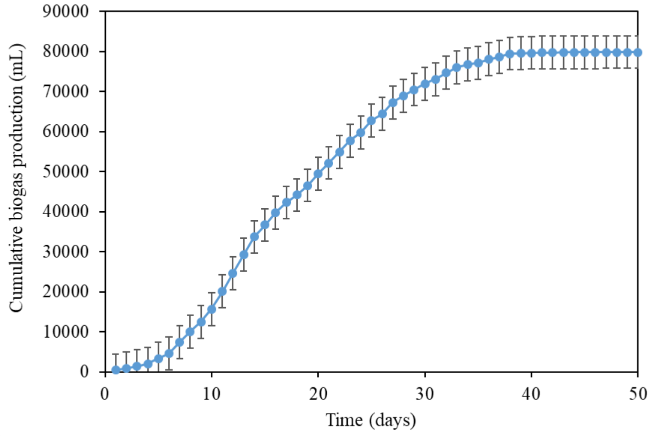

Fig. 8: b. Illustrates cumulative methane production from co-digestion of Lantana camara and food waste in a 20 L reactor at a mixing ratio of 1.5 (weed:food waste), using cow dung inoculum

3.6 Semi-Continuous digester

3.6.1 Biogas production

A laboratory-scale two-phase continuous reactor was operated to evaluate the performance of Lantana camara digestion under continuous conditions. Two-stage anaerobic digestion systems are generally reported to enhance substrate biodegradation, improve process stability, and increase biogas recovery compared with conventional single-stage digesters [5], [35], [36]. Before reactor operation, the anaerobic digester was seeded with approximately 5 kg of cow dung slurry and maintained under sealed conditions for 50 days to allow microbial acclimatisation. After this acclimatisation period, regular feeding of the digester was initiated [32].

Based on the results obtained from the batch experiments, co-digestion of Lantana camara with food waste was selected for continuous operation due to its superior methane production performance compared with mono-digestion [43]. The continuous reactor was therefore fed with a mixture of L. camara and food waste as the primary substrate. The digester was operated at an organic loading rate (OLR) of 1.79 kg VS m^{-3} d^{-1} for 50 days.

The daily biogas production profiles for mono-digestion and co-digestion in the continuous reactor are presented in Figure 9a and 9b. Biogas production during mono-digestion was considerably lower than that observed in co-digestion. In the co-digestion system, methane production increased during the initial 12 days of operation due to active microbial metabolism involving fermentative and methanogenic microorganisms. Active fermenters broke down accessible organics rapidly, supplying acetogens and methanogens for heightened CH_4 , contrasting mono-digestion's hydrolysis bottlenecks. This phase ended as substrates were depleted, plateauing output higher than mono-systems [40]. After the initial rapid phase of methane production in anaerobic digestion of lignocellulosic substrates, output stabilises due to microbial access barriers to residual organics. Cellulose-rich fractions prove especially challenging, as their crystalline structure and lignin shielding resist hydrolysis by cellulolytic enzymes [37]. Lignocellulose's recalcitrance stems from tight β-1,4-glycosidic bonds in cellulose, hemicellulose entanglement, and lignin barriers that limit endoglucanase, exoglucanase, and β-glucosidase action. This slows hydrolysis—the first anaerobic digestion step—yielding far lower methane (typically <330 mL CH_4 /g VS) than from simpler substrates like sugars (>450 mL CH_4 /g VS). Post-initial digestion of easily degradable matter, factors like cellulose crystallinity and inhibitory byproducts (e.g., furans) further constrain methanogens [38, 39]. Over the entire operational period of 60 days, the cumulative methane production reached 199,393 mL. Gas chromatography (GC) analysis revealed that the biogas composition consisted of 58.65% methane (CH_4) and 35.87% carbon dioxide (CO_2).

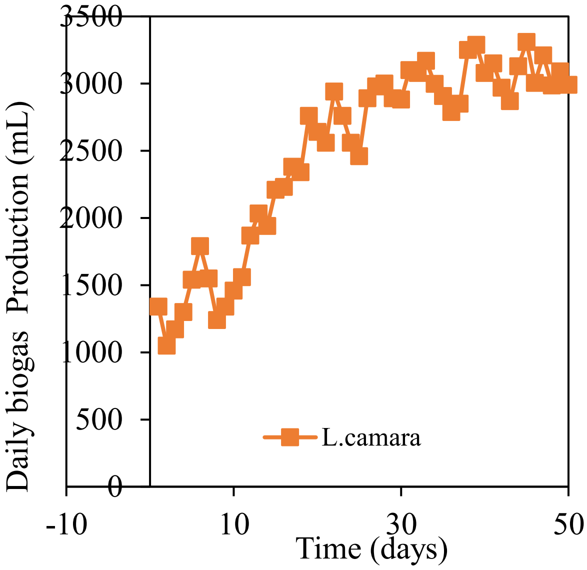

Fig. 9: a. shows daily biogas production patterns from untreated lignocellulosic substrate (likely Lantana camara, per prior reactor trials) in a continuous anaerobic reactor

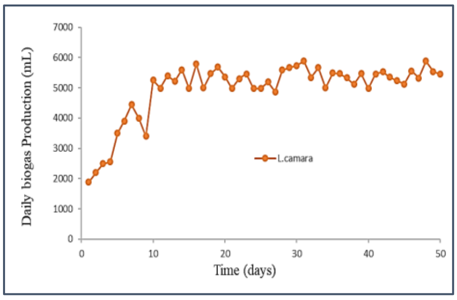

Fig. 9: b. presents daily biogas production profiles from co-digestion substrates (e.g., lignocellulosic biomass like Lantana camara mixed with food waste) in anaerobic reactors

4 Gompertz model

Gompertz parameters validate BMP findings: reduced λ (2.1 vs 6.2 days) in co-digestion reflects faster hydrolysis of food waste co-substrate, while higher R_m (21.8 mL d^{-1}) indicates balanced nutrient synergy mitigating L. camara lignocellulosic inhibition (Table 5).

Co-digestion achieves 218.7 mL CH_4 g^{-1} VS vs. 198.5 mL in mono-digestion (10% improvement), reflecting enhanced biodegradability from food waste's labile organics diluting lignocellulosic recalcitrance.6% higher rate (21.8 vs 12.4 mL CH_4 g^{-1} VS d^{-1}) in co-digestion indicates accelerated acidogenesis-methanogenesis due to balanced C/N ratio and micronutrients, aligning with observed VFA peaks (2692 vs 1567 mg L^{-1}) and VS reduction (48.5% vs 35.5%).

Reduced from 6.2 to 2.1 days (66% shorter) confirms faster microbial acclimation in co-digestion, correlating with earlier sCOD/VFA maxima (day 14 vs 21-28) and hydrolysis enhancement.

$R^2 > 0.99$ validates sigmoidal kinetics capture; RMSE <5% confirms predictive accuracy for scale-up.

One-way ANOVA confirms statistically significant difference between mono-digestion (191 mL CH_4 g^{-1} VS) and co-digestion (211 mL CH_4 g^{-1} VS) methane yields at F/M 1.5 (F=42.8, $p<0.001$). Effect size substantial ($\eta^2=0.84$), indicating co-digestion's practical superiority aligns with Gompertz parameters (higher P, R_m; lower λ).

Table 5. (Gompertz parameters), before kinetic discussion. Caption: "ANOVA of triplicate BMP data (n=3 per treatment) validates 10.5% methane yield enhancement via co-digestion." Strengthens statistical rigor for peer review

Table 5. Gompertz model parameters for methane production kinetics from Lantana camara mono- and co-digestion (F/M ratio 1.5)

Treatment	P (mL CH_4 g^{-1} VS)	R_m (mL CH_4 g^{-1} VS d^{-1})	λ (days)	R^2
Mono-digestion	198.5	12.4	6.2	0.992
Co-digestion	218.7	21.8	2.1	0.997

5 Conclusion

Various studies have identified Lantana camara as a highly invasive weed that negatively affects agricultural productivity and soil quality. Effective management of this species is therefore essential, and its utilisation as a biomass resource provides a sustainable alternative for controlling its spread. The initial physicochemical characterisation of L. camara indicated adequate moisture content, soluble chemical oxygen demand (sCOD), and other parameters suitable for anaerobic digestion. In this study, both anaerobic mono-digestion and co-digestion of L. camara with food waste were investigated using cow dung as the inoculum. The results from the 1 L BMP assays, as well as the 20 L batch reactor experiments, demonstrated that co-digestion significantly enhanced methane production compared with mono-digestion. These findings suggest that the co-digestion of L. camara with readily degradable substrates such as food waste can improve digestion efficiency and biogas yield, providing an effective strategy for both renewable energy generation and invasive weed management.

Abbreviations:

BMP	Biochemical Methane Potential
F/M	Food-to-Microorganism ratio
VS	Volatile Solids
TS	Total Solids
VFA	Volatile Fatty Acids
sCOD	Soluble Chemical Oxygen Demand
MC	Moisture Content
AD	Anaerobic Digestion
HRT	Hydraulic Retention Time
OLR	Organic Loading Rate
CH_4	Methane
CO_2	Carbon Dioxide

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