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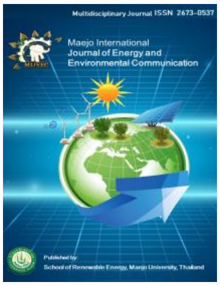

ARTICLE

# Revealing sustainable energy opportunities through the integrated use of *Canna indica* biomass and buffalo manure for biogas generation

Macdonald Tatenda Muronda[1] and Obey Gotore[2,]*

[1]Department of Water Science Engineering, Bu Ali Sina University, Hamedan 65178-38695, Iran
[2]Department of Biological Environment, Graduate School of Bioresource Sciences, Akita Prefectural University, Shimoshinjo-Nakano, Akita 011-0195, Japan

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## ABSTRACT

Mature *Canna indica* L., contains significant percentages of hemicellulose (21.6±0.22%) and lignin (20.14±0.13%), showing its high potential as a biogas source. This study explores the potential of using *C. indica* biomass harvested from waterlogged clay areas for biogas production. The research focuses on optimizing the anaerobic co-digestion process with swine dung through varying calcium oxide (CaO) pretreatment concentrations during a 45-day experiment. CaO pretreatment significantly enhances biogas yield, with 2% CaO yielding the highest biogas production at 8024.10 mL. Methane concentration analysis reveals that higher CaO concentrations, notably 2% and 3%, accelerate methane production, indicating an optimal CaO concentration of around 2% for maximizing methane yield. This study outperforms others in anaerobic co-digestion, achieving a methane concentration of 64.93%. Data on *C. indica* at different CaO concentrations as a substrate underscores the need for precise CaO tuning for optimal methane production. The findings open avenues for sustainable waste management and renewable energy production, hinting at promising developments in energy solutions through optimized anaerobic co-digestion processes using *C. indica* and buffalo dung.

## 1. Introduction

Wetland ecosystems are critical components of our planet's ecological tapestry, revered for their unique hydrological dynamics, biodiversity harboring capabilities, and crucial roles in carbon sequestration and water purification. However, these precious habitats face mounting pressures due to urbanization, pollution, and climate change, necessitating innovative approaches to their conservation and sustainable utilization (Gotore et al., 2019). In this context, the intersection of wetland conservation and renewable energy generation presents a promising avenue for addressing ecological and energy challenges (Gotore et al., 2020). As the global imperative for sustainable wastewater treatment grows, wetlands emerge as a viable, economical, and eco-friendly solution. Plants serve as silent yet crucial engineers in this intricate ecosystem, transforming wastewater nutrients into biomass (Sharma et al., 2021). Certain plant species, particularly adapted to challenging environmental conditions, excel in nutrient uptake and robustness, making them ideal candidates for this living filtration system. Based on literature sources, *Phragmites australis*, *Typha latifolia*, and *Cyperus papyrus* are frequently employed in wetland areas. However, *Canna indica* is a promising candidate for use in engineered wetlands (Karungamye, 2022).

One standout is *C. indica* L., a species notable for its efficacy in removing nitrogen and phosphorous and its resilience to chemicals and metals. Especially in tropical climates, where the

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* Corresponding author.
*E-mail address:* gotore_o@akita-pu.ac.jp (Gotore. O)

conditions amplify their efficacy, these plants purify water and add aesthetic value with their vibrant inflorescences. However, despite their potential, more is needed to know how these species cope with specific challenges like low $O_2$ availability and high external ferric iron (Nguyen et al., 2021). One significant advantage of using *C. indica* over the commonly used *P. australis* is its rapid growth and abundant biomass production. Quick-growing plants with extensive root systems are beneficial for promoting nitrifying bacteria, which enhances the nitrification process by expanding the biofilm surface area. Additionally, *C. indica* requires 3–5 times more water than standard wetland vegetation and offers aesthetic value due to its blooming flowers (Kaur et al., 2021). In pollution abatement and reducing greenhouse gas emissions, *C. indica* is more effective than *C. alternifolius* and *P. australis*. The plant's robust root system enhances pollutant removal and improves aerobic conditions in constructed wetlands. *C. indica* has superior root characteristics and is highly pollution-resistant compared to other species (Sharma et al., 2021). Its aerenchyma tissue optimizes nitrification by directing oxygen to the roots. These findings offer ways to boost wetland efficiency and sustainability, paving the way for converting biomass to biogas.

Biomass is an eco-friendly, renewable energy derived initially from solar power (sunlight). It can be replenished relatively quickly through the growth of plants or algae (Chuanchai et al., 2019). Common sources like trees, crops, and urban waste are abundant and can be sustainably managed (Nong et al., 2022a,b). In the quest for sustainable biogas production through (AD), the focus is on utilizing non-food competitive biomass types like agricultural waste, local biomass, and municipal or industrial bio-waste (Nong et al., 2022c). *C. indica*, a wetland plant, is an intriguing candidate for biogas feedstock, offering a balanced blend of environmental remediation and sustainable power generation (Nong et al., 2020). Biogas is a byproduct of bacterial decomposition of organic matter and can be generated from waste management systems or plant matter. Various biodegradable substances, such as green waste, animal by-products, sewage sludge, and manure, can be used for biogas production.

Typically, biogas comprises 55-60% methane ($CH_4$), a range of 30-35% carbon dioxide ($CO_2$), a small amount of water vapor, and other tiny amounts of gases. Only the methane component is helpful for energy generation, and its concentration can vary based on the raw materials used (Wannapokin et al., 2018). This makes biogas a highly potent fuel source. Lignocellulosic biomass holds promise for biogas production through AD. Its breakdown depends on its chemical makeup, like cellulose and lignin. Biomass mainly consists of complex polymers degraded in a four-step AD process. Hydrolysis is the slowest step due to these complex polymers. Pretreatment prepares the biomass for easier microbial breakdown, improving biogas yield. This can be physical, chemical, or biological and should be mild to avoid sugar degradation. Also, co-digestion outperforms mono-digestion for biogas production by providing better nutrient balance, moisture, and buffer capacity. It also dilutes toxins and boosts biodegradability. Typically, co-digestion can increase biogas yields up to 3.46 times due to synergistic effects and added nutrients (Tamilarasan et al., 2017). Chuanchai and Ramaraj (2018) suggested that co-digestion of buffalo dung could improve biogas production as a co-substrate. The main objective of this work was to enhance the anaerobic co-digestion of *C. indica* and buffalo dung by focusing on chemical pretreatment for biogas enhancement.

## 2. Material and methods

### *2.1 Preparation of raw materials, collection, and experimental location*

*Canna indica* L. samples were harvested from waterlogged clay areas in the open field of the Faculty of Fisheries Technology and Aquatic Resources at Maejo University, Chiang Mai, Thailand. Following collection, mature plants underwent a cleaning process to remove soil and clay and were then cultivated in water-saturated sand until new growth appeared. These new plants were then cleaned to remove sand before being mechanically processed into small particles and stored at 4 °C for future use. Buffalo dung sourced near the Energy Research Center (ERC) was also utilized in the experiment, which took place at the ERC under the supervision of founding professor Dr. Natthawud Dussadee during the author's internship at Maejo University.

### *2.2 Pretreatment*

The substrate (mechanically processed small particles) was pretreated using control (0%), 1%, 2%, and 3% (v/v) calcium oxide (CaO) for 48 h. This study was conducted to determine the optimal and efficient pretreatment condition for enhanced biogas production. The sample was collected *C. indica* and pretreated, and swine dung was associated with co-digestion fermentation processes to produce biogas.

### *2.3 Experimental setup and procedure for biogas production*

The layout of the anaerobic co-digestion and biogas production process is presented in Figure 1. Biogas was generated using a batch system with laboratory-scale digesters created from 1-L water tanks, outfitted with a 1000 mL cylinder for gas collection, measurement, and a feeding port.

**Figure 1.** Layout of anaerobic co-digestion and biogas production process

This setup was secured with a rubber stopper fitted with a pipe for biogas extraction. The experiment utilized *C. indica* and swine dung as co-substrates housed in Duran glass bottles of 1-L capacity, maintaining a functional volume of 800 mL (the procedure was adopted from Chuanchai and Ramaraj, 2018).

To foster anaerobic conditions, the bottles were purged with nitrogen gas. Each assay incorporated 80 mL of inoculum, 200 g each of crushed *C. indica*, and fresh swine dung, topped up with double-distilled water. The assessments were executed in triplicate to ensure precise data collection, and all used a lab-scale fermenter for 45 days. It was stored at 4 °C in an oxygen-free environment inside a cooler to restrain unwanted methane production from the inoculum. The inoculum was utilized from the working biogas feed batch system. This setup ensured the experiment's integrity by minimizing external influences on the methane production process.

### *2.4 Analytical methods*

To characterize the solid contents, total solids (TS), volatile solids (VS), and pH assessments were conducted in line with the procedures detailed in the Standard Methods for the Examination of Water and Wastewater, as outlined by the APHA (2005). The raw materials' moisture content was ascertained per the ASTM Standard D 4442-07 guidelines. Both proximate and ultimate analyses were conducted to study the samples further. A comprehensive assessment of total fat, ash, moisture, and fiber contents was undertaken following the standard method stipulated by APHA. Moreover, a BIOGAS 5000 analyzer from Geotech was used to gauge the composition of the resultant biogas, focusing on methane, carbon dioxide, and oxygen components ($CO_2$ and $O_2$ data were not shown in this article).

### *2.5 Statistical analysis*

The data gathered was processed through SPSS version 16.0 for analysis. An ANOVA test was carried out to pinpoint variations in FA content. For post hoc multi-comparisons, Tukey's Studentized range test was deployed.

## 3. Results and discussion

### *3.1 Plant distribution and genetic diversity*

The *C. indica*, belonging to the Cannaceae family and the Canna genus, is renowned for its distinct morphological characteristics. As a perennial plant, it showcases a life cycle extending over several years, continuously gracing environments with its presence. It boasts a bulbous nature, suggesting a swollen or enlarged configuration often associated with storage organs that house nutrients. Furthermore, it falls under the herbaceous category, indicating that its stem is relatively soft, not woody, and generally green (Jiang et al., 2014). The plant is adorned with vibrant, herbaceous flowers, accentuating its aesthetic appeal and underscoring its botanical identity grounded in a harmonious blend of utility and visual delight (Sharma et al., 2021). This blend of attributes paints a picture of a robust plant in its survival strategy and is beautiful in its presentation, bringing to life gardens and landscapes with a splash of color and vitality.

*C. indica* L, or Indian Shot, is a perennial plant native to much of South America, Central America, and the Caribbean. Over time, its cultivation has spread to many other parts of the world, where it is used ornamentally and for various other purposes. Below is provided a discussion about the distribution and genetic diversity of *C. indica* L:

Distribution: Native Range

- South America: Predominantly found in the tropical and subtropical regions.
- Central America and the Caribbean: Commonly found in these regions, especially in wet and marshy areas.

Introduced Range:

- North America: Found in gardens and landscapes, particularly in the southeastern US.
- Europe: Mainly cultivated as an ornamental plant.
- Asia: Cultivated in gardens and naturalized in several countries, including India.
- Africa: Found in several African countries, mainly in gardens and as an invasive species in some areas.
- Australia: Cultivated as an ornamental plant and also identified as an invasive species in certain regions.

Genetic Diversity:

*C. indica* L has a complex genetic structure due to its long history of cultivation and hybridization with other species in the genus Canna. Here are some points regarding its genetic diversity:

- Hybridization: The species has been extensively hybridized, resulting in different cultivars with varied characteristics. This hybridization has contributed significantly to the genetic diversity of the species.
- Polyploidy: *C. indica* L is known to exhibit polyploidy, where organisms have more than two paired sets of chromosomes. This trait can potentially increase genetic diversity within populations.
- Seed dispersion: The plant has hard seeds that facilitate long-distance dispersion, potentially aiding genetic diversity by mixing gene pools over large areas.
- Cultivars and varieties: Many cultivars and varieties of *C. indica* L. have been developed through selective breeding. This selective breeding has enriched the genetic diversity in the cultivated populations.
- Conservation and studies: To truly understand the genetic diversity of *C. indica* L., more studies employing modern genomic tools are required. There might be localized populations with unique genetic traits, and conserving these populations can help preserve the species' genetic diversity.

*C. indica* L is widely distributed across various continents due to its ornamental value and ability to grow in different environments. It has considerable genetic diversity from natural evolution, hybridization, and selective breeding (Kaur et al., 2021). The *C. indica* is cultivated for its rhizomes between 0 and 2500 meters above sea level, requiring 500 mm/year of precipitation. It has two cultivation durations: 180 days for fresh consumption and 550 days for industrial starch extraction, with a plant arrangement of 80 cm between rows and 50 cm between plants, aiming for a yield between 25.5 and 35.0 tons per hectare (Cereda and Vilpoux, 2023).

### *3.2 Main components of C. indica biomass*

The detailed breakdown of the *C. indica* biomass showcases its considerable potential as a biogas source (Table 1). The composition encompasses a hemicellulose percentage of 21.6±0.22, paired with a lignin content of 20.14±0.13%, reflecting a significant presence of these complex polymers. Moreover, a minimal fraction of acid-insoluble ash quantified at 0.8±0.02%, carbohydrates the primary constitution of organic components. The high percentage of total solids, accounting for 97.7±0.45% of the mass, forms a substantial base for biogas production, which often relies on solid biomass feedstock. Significantly, the volatile solids component, which constitutes 79.9±0.37% of the biomass, indicates the presence of materials that can be vaporized at high temperatures, a vital characteristic in anaerobic digestion processes commonly utilized in biogas production.

**Table 1.** Components of *C. indica* biomass

| **Parameters** | **Biomass (%)** |
|---|---|
| Total solids | 97.7 ± 0.45 |
| Volatile solids | 79.9 ± 0.37 |
| Protein | 21.8 ± 0.22 |
| Fat | 4.3 ± 0.14 |
| Carbohydrate | 13.4 ± 0.05 |

Moreover, the substantial protein content of 21.8±0.22% suggests that the biomass could facilitate a high yield of biogas, given that proteins can be converted to gases like methane and carbon dioxide during anaerobic digestion. The fat and carbohydrate contents, representing 4.3±0.14% and 13.4±0.05% of the biomass respectively, further complement this potential, as they are known to be excellent substrates for biogas production, providing necessary carbon sources that microbes can feed on to produce biogas (Van Tran et al., 2022).

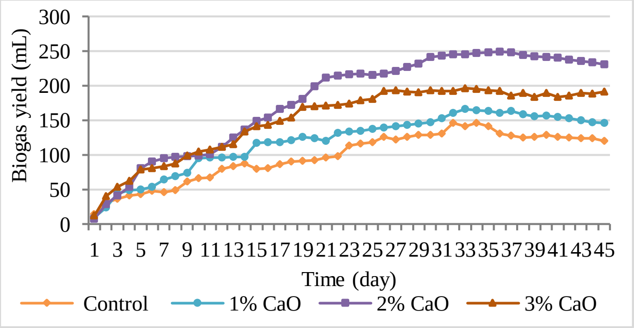

**Figure 1.** Daily biogas yield

### *3.3 Pretrment effect of fermentation and biogas yield*

In discussing the effect of pretreatment on biogas yield, it is evident that optimizing the biogas production process is a critical facet that demands substantial attention (Unpaprom et al., 2019). Various pretreatment methods such as physical, chemical, and biological approaches stand pivotal in enhancing feedstocks' digestibility and fermentation efficiency, including those from *C. indica*. The data presented provides a meticulous day-by-day analysis of biogas yield over 45 days under various conditions including control and different concentrations of CaO pretreatment (1%, 2%, and 3%) and buffalo dung. As can be seen, pretreatment with CaO substantially impacts the biogas yield, significantly altering the daily and total production values compared to the control group.

Looking deeper into the data, there is a noticeable trend where increasing concentrations of CaO pretreatment generally lead to an increase in daily biogas yield as the experiment progresses. The most significant rise is observed in the 2% CaO group, achieving the highest total biogas yield at the end of the 45 days, at a staggering 8024.10 mL. This showcases the potential for enhanced biogas production through the strategic use of chemical pretreatment methods. Furthermore, observing the initial days, it is apparent that the control group had a better start than groups with CaO pretreatment, suggesting that pretreatment might have somewhat inhibited the initial biogas production. However, as days progressed, the tables turned notably with the CaO groups overtaking the control group substantially, indicating the pretreatment's effectiveness in the long run.

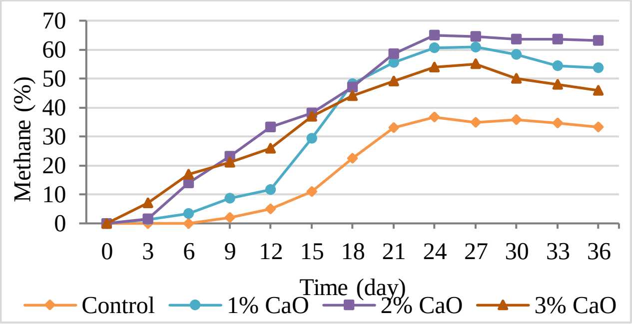

**Figure 2.** Methane concentrations

### *3.4 Methane concentration on biogas with different ratios of pretreatment*

The initiation stage of extracting cellulose from biomass hinges on a pivotal pretreatment step that efficiently breaks down lignin barriers, using the least energy and keeping costs low (Wannapokin et al., 2018). The presented data in Figure 2 discloses the progression of methane concentration in biogas over 45 days, taking into account various pretreatment ratios of *C. indica* with buffalo dung under different conditions: control and with CaO pretreatments of 1%, 2%, and 3%. At the outset of the observation period, all groups registered a zero concentration of methane, showing that the biogas production had yet to initiate. Over the initial days, it is observable that the pretreated groups demonstrated a discernible acceleration in methane production compared to the control group. Notably, the groups with higher concentrations of CaO exhibited a more rapid increase, showcasing the positive impact of CaO pretreatment on initiating and escalating the methane production process.

As we progress through the data, a notable spike in methane concentration is witnessed around day 9 for the 2% and 3% CaO groups, indicating that a higher concentration of CaO can facilitate a quicker onset of substantial methane production. Interestingly, the 3% CaO group did not maintain its early lead; instead, it was surpassed by the 2% CaO group from day 12 onward, suggesting an optimal concentration of CaO for enhancing methane yield, which is around

2%. Analyzing the data from day 15 to 36, it is apparent that all groups experienced a steady increase in methane concentration, with the 2% CaO group consistently leading, followed closely by the 3% CaO group. The 1% CaO group also showed a consistent rise but was always a step behind the 2% and 3% groups, showcasing a dose-dependent effect of CaO pretreatment up to a certain point.

**Table 2.** Methane concentration comparison of different anaerobic co-digestion

| Substrate | Co-substrate | Methane (%) | Reference |
|---|---|---|---|
| Food waste and | Domestic wastewater | 56 | Chan et al. (2018) |
| Water hyacinth | Cow dung | 56.40 | Uche et al. (2019) |
| Water hyacinth | Pig and poultry droppings | 64.92 | Okewale and Adesina (2019) |
| Waste fruits | Waste vegetables | 63.71 | Saetang and Tipnee (2022) |
| Kitchen waste | EM | 62.2% | Minza et al. (2021) |
| *C. indica* (1% CaO) | Buffalo dung | 60.92 | This study |
| *C. indica* (2% CaO) | Buffalo dung | 64.93 | This study |
| *C. indica* (3% CaO) | Buffalo dung | 55 | This study |

Table 2 delineates the methane concentrations resulting from various anaerobic co-digestion processes involving different combinations of substrates and co-substrates, referenced from several studies from 2018 to the present study under discussion. Firstly, looking at the data, it is apparent that different combinations of substrates and co-substrates result in methane concentrations ranging from 55 to almost 65%. This wide range suggests the complex interplay of the materials used in influencing the final yield of methane, indicating that small changes in the substrate or co-substrate can significantly impact the outcomes (Unpaprom et al., 2021).

Regarding the studies referenced, the comparability of the results obtained in this study with other referenced works is worth noting. Particularly, the co-digestion involving 2% CaO pretreated *C. indica* and buffalo dung from the present study stands out with a notable methane concentration of 64.93%, which is in close quarters with the highest yields reported in other referenced works, showcasing it as a highly efficient combination in terms of methane production. The data involving *C. indica* at different CaO concentrations as a substrate portrays a fluctuating trend in methane yield.

While an increase in CaO concentration from 1% to 2% led to an enhancement in methane concentration, a further increase to 3% of CaO recorded a decline, falling even below the 1% CaO concentration's yield. This possibly hints at an optimal CaO concentration between 1% and 3% for *C. indica* and buffalo dung co-digestion, advocating for a detailed exploration into fine-tuning the CaO concentration to attain the most favorable methane yield. Also noteworthy is the respectable methane yield derived from the co-digestion of waste fruits and waste vegetables according to Saetang and Tipnee (2022), emphasizing the potential for high methane yields from waste materials and by extension underscoring the prospects of anaerobic co-digestion as a sustainable approach to managing organic wastes while generating energy.

It is pivotal to recognize the diversity in the combinations studied, encompassing a variety of organic wastes including food and kitchen waste, animal dung, and aquatic plant biomass. This underlines the versatile nature of anaerobic co-digestion processes in accommodating various organic materials as substrates (Junluthin et al., 2021; Souvannasou et al., 2021a,b). Therefore, this table illuminates the promising potential of anaerobic co-digestion as a strategy for waste management and a substantial source of renewable energy through methane production. It reflects the necessity for further research into optimizing the conditions and material combinations for anaerobic co-digestion to enhance methane yields further, potentially steering towards a sustainable and greener future. The data could also pave the path for scaling up the anaerobic digestion process, focusing on utilizing locally available waste resources to create renewable energy solutions tailored to specific geographic and demographic contexts.

## 4. Conclusion

The experimental study manifests *Canna indica* L's considerable potential as a pivotal resource in biogas production. Through well-calibrated and strategically utilizing chemical pretreatment methods, particularly employing 2% CaO, a significant enhancement in biogas and methane yield is achievable, underscoring the plant's high carbohydrate, fat, and protein content. The findings illuminate the path towards eco-friendly energy solutions, spotlighting anaerobic co-digestion as a viable strategy in waste management and renewable energy production. In a world steering towards sustainable and green solutions, this research stands significant, underscoring the tangible potential embedded in plant biomass and organic wastes, urging a deeper dive into anaerobic co-digestion research. The study reflects an encouraging prospect of scaling up these processes, converging towards creating tailored renewable energy solutions that resonate with specific geographic and demographic nuances. It is a significant stride in embracing the green revolution, beckoning a future where energy production is harmoniously aligned with nature, fostering a synergy that is sustainable and yielding in the long run.

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## Conflict of Interest Declaration

The authors assert that no conflicts or personal affiliations might be construed as impacting the outcomes shared in this research.

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