

## Enhancing hydrolysis from cow dung manure using ultraviolet-lamps for efficient biogas production

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### ABSTRACT

Anaerobic digestion (AD) of livestock waste is a sustainable pathway for renewable energy production, yet methane yield is often limited by the slow hydrolysis of lignocellulosic material. Pretreatment methods have been proposed, but many are costly or environmentally burdensome. This study investigated ultraviolet light irradiation as a novel, low-energy pretreatment to enhance the methane production from cow dung. Fresh cow dung was exposed to UV light at 254 nm and intensity and for 0 (control), 30, 60, 90, 120, 150, and 180 minutes before undergoing 35-day anaerobic digestion at 37 °C. Methane yield and chemical oxygen demand (COD) removal were monitored. Results revealed a significant effect of UV exposure duration on both methane yield ( $p < 0.05$ ) and COD removal ( $p < 0.05$ ). Optimal performance occurred at 120 minutes (methane yield:  $126 \pm 28.3$  mL/day; COD removal: 58.9%), representing a more than twofold improvement compared to the control. Overexposure ( $\geq 150$  minutes) decreased methane yield and COD removal, likely due to the generation of inhibitory by-products such as furans or phenolic compounds. These findings suggest that UV light pretreatment, when optimised, can partially overcome the hydrolysis limitations in AD. However, the study did not assess microbial dynamics, chemical intermediates, or scale-up feasibility, which remain critical gaps for future research.

**Keywords:** UV light pretreatment, anaerobic digestion, cow dung, methane yield, COD removal.

### INTRODUCTION

The increasing global demand for renewable energy has intensified interest in biogas production from organic waste, offering a dual benefit of sustainable energy generation and effective waste management (Alengebawy et al., 2024). Biogas, a clean and renewable energy source, presents a viable alternative to fossil fuels (Sharma et al., 2025). In Tanzania and other agro-based nations across Africa, livestock farming generates substantial quantities of cow dung, a rich resource for biogas production through anaerobic digestion (AD) (Sibanda and Uzabakiraho, 2024).

Anaerobic digestion is a biological process in which microorganisms break down organic matter in the absence of oxygen, proceeding through four primary stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Jacob et al.,

2025). Among these stages, hydrolysis is widely recognised as the rate-limiting step, especially when processing complex organic substrates.

This limitation is often due to the formation of toxic by-products, such as complex heterocyclic compounds, which inhibit subsequent steps as well as result in reduced biogas yield and methane content. To address this challenge, various pretreatment methods have been explored to improve hydrolysis efficiency.

While conventional pretreatment methods, encompassing mechanical, thermal, and chemical approaches, have been extensively explored to enhance anaerobic digestion efficiency by improving substrate biodegradability, each carries specific advantages and inherent limitations, including energy intensity, potential for inhibitor formation, or substantial operational costs (Rahmati et al., 2020). In the pursuit of more effective

and sustainable solutions, advanced oxidative processes, particularly various forms of radiation, have gained attention. For instance, high-energy ionising radiation, such as gamma radiation often sourced from Cobalt-60, has been investigated for its capacity to modify biomass structure and improve digestibility (Wiszumirska et al., 2023). Gamma irradiation is utilised in various industrial applications like food sterilization (Kakatkar et al., 2024) and wastewater decontamination. Its direct, economically viable application for large-scale biogas production from organic waste is dubious, especially considering environmental and cost factors (Haroun et al., 2020). The broader context of managing radioactive waste and its associated environmental and economic challenges remains a significant concern (Rana et al., 2020).

Consequently, research has increasingly focused on less hazardous and potentially more accessible radiation-based technologies, such as ultraviolet irradiation and ultrasonic pretreatment. Ultrasonic pretreatment effectively enhances solubilization (Dębowski et al., 2023) and biogas production (Liu et al., 2021) by facilitating the breakdown of polymeric matters as well as chemical bonds within the substrate, and can reduce the hydrolysis phase period (Rashvanlou et al., 2021). Yet, ultrasonic methods can be energy-intensive (Paul et al., 2023), while they can significantly increase biogas yield, the energy balance must be carefully considered, as some applications may require more energy than the additional biogas generated (Witaszek et al., 2020), indicating a potential negative energy balance.

Recent advancements in ultraviolet light radiation technology offer a novel solution. UV light offers a promising approach for degrading complex organic matter through both advanced oxidation processes and direct photolysis. When applied in advanced oxidation processes, it effectively breaks down dissolved organic matter (Gao et al., 2020), often by generating highly reactive hydroxyl radicals ( $\text{HO}^\bullet$ ) and other radical species (Choi and Chung, 2020; El-Gawad et al., 2023). For instance, a 275 nm UV-LED has been shown to significantly remove humic acid in UV/chlorine AOPs, primarily through radical-mediated degradation (Gao et al., 2020), and UV-LED-driven AOPs simultaneously remove microcontaminants in wastewater (Miralles-Cuevas et al., 2021). Beyond radical-based mechanisms, UV light can directly photolysis organic molecules. This involves the direct absorption of UVC photons, leading to the breaking of

chemical bonds and molecular fragmentation, without necessarily relying on external oxidants or the subsequent generation of radical species. Examples of this direct degradation include the photolysis of volatile organic compounds like toluene, where the absorbed UV-C energy cleaves covalent bonds, leading to photo-degradation (El-Tawary, 2022). Similarly, certain polymers undergo direct backbone cleavage upon UV exposure, forming smaller molecules through a self-immolative mechanism (de Gracia Lux et al., 2012).

Studies on the interaction of organic molecules with vacuum ultraviolet photons also demonstrate direct fragmentation dynamics, where VUV photon absorption directly breaks molecules (Haitjema et al., 2021). The direct photolysis of chlorophenols in aqueous solutions by specific ultraviolet light further illustrates this mechanism, with observed degradation rates attributable to direct photon absorption (Matafonova et al., 2011).

This capacity for direct bond scission and fragmentation highlights UVC's potential to break down larger, recalcitrant molecules into smaller, more manageable forms. This positions UVC lamp as a promising and sustainable option that addresses many of the drawbacks associated with conventional pretreatment methods, including the high energy requirement and lower light efficiency of traditional UV light (MacIsaac et al., 2023), and the generation of chemical waste. Therefore, this study investigated the application of UV-light technology as a novel pretreatment method to enhance the hydrolysis step in the anaerobic digestion of cow dung, with the goal of improving chemical oxygen demand removal and boosting overall biogas production efficiency. The authors hypothesise that the application of UV-light technology as a pretreatment method to cow dung will significantly enhance the hydrolysis step in anaerobic digestion, leading to improved chemical oxygen demand removal and increased biogas production efficiency compared to untreated cow dung.

## MATERIALS AND METHODS

### Experimental setup

This study was conducted to evaluate the effect of UV-light pretreatment on anaerobic digestion (AD) of cow dung under mesophilic

conditions. A laboratory-scale, batch-mode experiment was carried out using a 270 mL glass bottle with a working volume of 200 mL, serving as anaerobic digesters. Each digester was sealed with rubber bungs to maintain anaerobic conditions. Biogas was collected in plastic gas-collection bags, which were tightly sealed to prevent leakage, as shown in Figure 1.

Fresh cow dung was collected from a local dairy farm in Arusha, Tanzania. Inoculum was obtained from an active biogas plant at the same location. A mixing ratio of 3:1 (cow dung to inoculum) was used.

For the pretreatment process, 150 mL of cow dung was placed in Pyrex beakers and exposed to a 254 nm, 60 W lamp positioned 15 cm above the sample in a dark fume chamber. Exposure durations were for 30, 60, 90, 120, 150, and 180 minutes. A control group (0 min exposure) was also prepared and maintained. Each treatment, including the control, was performed in triplicate. The light intensity of lamp 20 W/m<sup>2</sup> hr was set up to be nearly firm throughout the experiments. After UV-light treatment, each sample was mixed with 50 mL of inoculum, resulting in a total working volume of 200 mL in the 270 mL digestion bottles. The digesters were incubated at 37 °C and agitated at 90 rpm for 35 days until no further biogas production was observed.

### Analytical methods

Before digestion, the cow dung substrate was analysed for pH, moisture content (MC), and volatile solids (VS). The total solid (TS) and VS contents were determined following the American Public Health Association (APHA, 2005) standard methods for water and wastewater examination. TS was measured by drying samples at 105 °C for 24 hours, followed by incineration at 550 °C for 2 hours in a furnace (model JFF 2000, NEYCRAFT, York, PA, USA). The methane content was determined volumetrically by passing the collected gas through 15% (w/v) KOH with 1% methylene red indicator, where a reduction in gas volume reflected methane content. It is important to note that this method provides an approximate estimation of methane content and does not offer the precise compositional analysis achievable with chromatographic techniques. Soluble chemical oxygen demand (sCOD) was determined using HACH low-range COD vials (0–1500 mg/L) and a DR6000 UV-Vis spectrophotometer, after

centrifuging digestate at 4.000 rpm for 10 minutes. sCOD removal was calculated as the percentage reduction from initial to final sCOD. Biogas production was measured using a gas-tight syringe and was recorded in millilitres per day (mL/day). In addition to initial cow dung characterisation, digestate samples were collected at the end of the 35-day digestion period for further analysis. Digestate was analysed for total solids (TS), volatile solids (VS), pH, total nitrogen (TN), total phosphorus (TP), potassium (K), and residual chemical oxygen demand (COD). TS, VS, and pH were determined as previously described. TN by Kjeldahl digestion, TP by colorimetry following acid digestion, and K by flame photometry. COD was measured using HACH COD digestion vials and spectrophotometry. All analyses for cow dung slurry (initial substrate) and digestate (post-AD) were conducted in triplicate (n = 3). Results are presented as mean ± standard error (SE).

### Statistical analysis

All statistical analyses and visualisations were performed using R software version 4.5.1 (R Core Team, 2025). Normality and homogeneity of variance were checked before analysis. The data from methane yield (MY) and chemical COD variables were imported using the read\_excel() function from the readxl package (Wickham and Bryan, 2023). To assess the effects of ultraviolet (UV) exposure on each response variable, a one-way analysis of variance (ANOVA) was conducted using the base R aov() function, with UV exposure as the fixed factor. Post-hoc mean separation was performed using Fisher's least significant difference (LSD) test via the LSD.test() function from the agricolae package (de Mendiburu, 2021), without adjustment for multiple comparisons. Treatment groupings were extracted and merged with summary statistics (mean, standard deviation, standard error, and sample size) calculated using summarise() and left\_join() from the dplyr package (Wickham et al., 2023).

For time-series visualisation, the duration (in days) variable was converted to numeric where necessary, and response trends over time were plotted using the ggplot2 package (Wickham, 2016).

Line plots included distinct shape markers for each treatment using the scale\_shape\_manual() function and were formatted with the theme\_classic() and theme() functions for clarity. High-resolution plots (600 dpi) were exported using

the `ggsave()` function. This integrated statistical workflow enabled a clear assessment of treatment effects and trends in response variables under different UV exposure conditions.

## RESULTS

### Digestate and characteristics

At the end of the 35-day digestion, significant differences were observed between the cow dung slurry and the digestate (Table 1). Total solids decreased from  $13.97 \pm 0.52\%$  to  $7.62 \pm 0.48\%$ , while volatile solids decreased from  $13.41 \pm 0.45\%$  to  $4.85 \pm 0.36\%$ , confirming extensive organic matter mineralisation. Digestate pH increased slightly ( $7.20 \pm 0.08$  to  $7.70 \pm 0.09$ ), while TN declined marginally ( $1.85 \pm 0.11\%$  to  $1.63 \pm 0.07\%$ ). Ammonium-N accumulated ( $2.250 \pm 121$  mg/L) due to protein degradation. The TP and K concentrations increased from  $950 \pm 43$  mg/kg to  $1.120 \pm 55$  mg/kg and  $2.240 \pm 97$  mg/kg to  $2.780$

$\pm 81$  mg/kg, respectively, indicating nutrient enrichment. COD decreased from  $38,200 \pm 1,320$  mg/L to  $15,700 \pm 606$  mg/L, aligning with COD removal trends (Figure 2).

### Cumulative methane yield

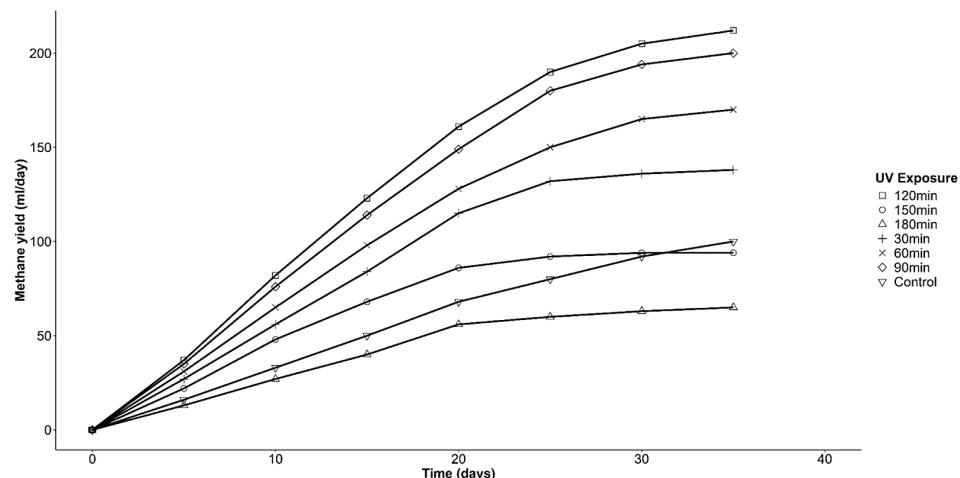
The results (Figure 1) present the daily methane yield (mean  $\pm$  SE mL/day) over a 35-day anaerobic digestion period under varying UV light exposure durations. Methane yield followed the trend: 120 min ( $126 \pm 28.3$ )  $>$  90 min ( $118 \pm 26.7$ )  $>$  60 min ( $101 \pm 22.5$ )  $>$  30 min ( $86 \pm 18.9$ )  $>$  150 min ( $63 \pm 12.8$ )  $>$  Control ( $54.9 \pm 12.8$ )  $>$  180 min ( $40.5 \pm 8.77$ ), with significant differences among treatments ( $p = 0.0239$ ).

### COD removal

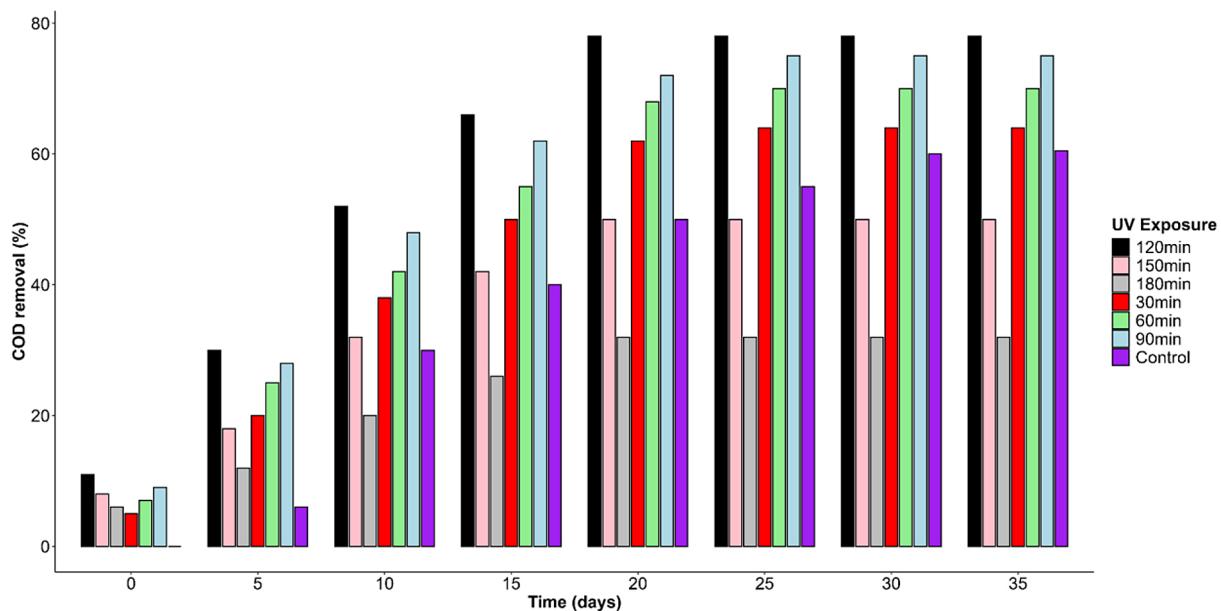
Chemical oxygen demand (COD) removal over a 35-day anaerobic digestion period differed significantly ( $p = 0.0368$ ) among the UV light exposure durations (Figure 2). The trend in COD

**Table 1.** Chemical characteristics of cow dung slurry (initial substrate) and digestate (post-AD), expressed as mean  $\pm$  SE ( $n = 3$ )

Parameter	Cow dung slurry (initial, mean $\pm$ SE)	Digestate (post-AD, mean $\pm$ SE)
TS (% FM)	$13.97 \pm 0.52$	$7.62 \pm 0.48$
VS (% FM)	$13.41 \pm 0.45$	$4.85 \pm 0.36$
pH	$7.20 \pm 0.08$	$7.70 \pm 0.09$
Total Nitrogen (TN, %)	$1.85 \pm 0.11$	$1.63 \pm 0.07$
Total Phosphorus (TP, mg/kg)	$950 \pm 43$	$1,120 \pm 55$
Potassium (K, mg/kg)	$2,240 \pm 97$	$2,780 \pm 81$
COD (mg/L)	$38,200 \pm 1,320$	$15,700 \pm 606$



**Figure 1.** Effect of UV light exposure on cumulative methane yield over 35 days from anaerobic digestion of cow dung (mean  $\pm$  SE,  $n = 3$ )



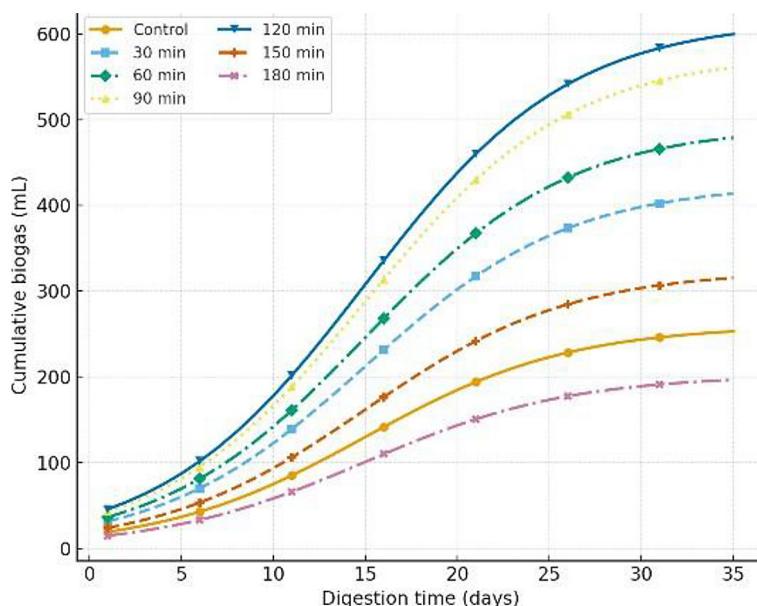
**Figure 2.** Efficiency of UV light exposure on daily COD removal calculated as % reduction from initial sCOD (mean  $\pm$  SE, n = 3)

removal (mean  $\pm$  SE %) was: 120 min (58.9  $\pm$  9.15) > 90 min (55.5  $\pm$  8.89) > 60 min (50.9  $\pm$  8.54) > 30 min (45.9  $\pm$  8.09) > 150 min (37.5%  $\pm$  5.86) > Control (37.7  $\pm$  8.41) > 180 min (24.0  $\pm$  3.64).

## Cumulative biogas yield

Cumulative biogas production exhibited the typical sigmoidal growth curve characteristic of anaerobic digestion processes (Figure 3). All treatments showed an initial lag phase during the first

few days, followed by a rapid increase in gas production between days 5 and 20, then gradually stabilised around day 30–35. The untreated control group achieved the lowest final cumulative yield ( $260 \pm 86.7$  mL), indicating the limited biodegradability of raw cow dung. In contrast, the UV-pretreated samples demonstrated significantly higher cumulative production, with the best results at 120 min exposure ( $615.8 \pm 202.0$  mL), closely followed by the 90 min treatment ( $576.0 \pm 189.3$  mL). Moderate pretreatments of 30 and 60 min also enhanced



**Figure 3.** Effect of UV light exposure on cumulative biogas production from anaerobic digestion of cow dung (mean  $\pm$  SE, n = 3)

production compared to the control, though to a lesser degree. Excessive UV exposure (150 and 180 min) reduced cumulative production to  $323.9 \pm 101.2$  mL and  $201.5 \pm 65.1$  mL, respectively.

## DISCUSSION

### Key findings

In this study, UV light pretreatment significantly influenced the methane production from cow dung anaerobic digestion, demonstrating a clear optimal range. A gradual increase in methane production was observed across all treated groups over time, following typical anaerobic digestion kinetics. The highest methane yields were achieved with moderate UV light exposures of 90 and 120 minutes. Conversely, shorter exposures (30 and 60 minutes) resulted in intermediate methane outputs, indicating only partial improvement in substrate digestibility. Notably, prolonged UV pretreatments (150 and 180 minutes) and the untreated control group exhibited significantly reduced methane yields compared to the optimal exposures. The pattern of chemical oxygen demand removal mirrored that of methane production, with increased efficiency under moderate UV exposure durations (60 to 120 minutes), and reduced removal at shorter, longer, and untreated conditions. The untreated control consistently showed the lowest performance for both methane yield and COD removal.

The chemical profile of the digestate reinforces the performance trends observed in methane yield and COD removal. The marked reduction in TS and VS demonstrates effective organic matter stabilisation under optimal UV pretreatment (90–120 minutes). The enrichment of ammonium-N indicates active protein hydrolysis and mineralisation, providing evidence of enhanced biodegradability of the substrate. Furthermore, the relative increases in TP and K reflect nutrient concentration due to volatile matter loss, positioning digestate as a valuable fertiliser product. These findings align with prior studies reporting that pretreatment-enhanced AD not only improves biogas yield but also produces nutrient-rich digestates suitable for soil amendment (Jacob et al., 2025; Orlando and Borja, 2020). The slight alkalinisation of digestate ( $\text{pH} \sim 7.7$ ) supports stable methanogenesis, as optimal microbial activity typically occurs under neutral to mildly alkaline conditions.

### Mechanisms of UV-light pretreatment on anaerobic digestion

The improved methane yields observed at 90 and 120 minutes of UV light exposure are primarily attributed to enhanced hydrolysis, where UV radiation likely disrupted complex organic structures within the cow dung matrix. UV-C light is known to break down compounds with high molecular weight, such as lignin, proteins, and polysaccharides into smaller, more bioavailable molecules, thereby improving biodegradability and enhancing subsequent methane production. This likely promotes microbial growth and enzymatic activity, particularly during the mid-phase of digestion. The increase in soluble organics accelerates their uptake and metabolism by microbial consortia, leading to more efficient COD degradation. By pre-fragmenting the particulate organic matter, UV light exposure likely reduced the energy required by microbial communities for extracellular enzymatic activity during hydrolysis.

Conversely, the poor performance at prolonged UV exposure (150 and 180 minutes) suggests that excessive irradiation may degrade critical organic molecules or produce inhibitory by-products that disrupt microbial communities and methanogenesis. The intermediate performance of the 30 and 60-minute treatments indicates a sub-threshold activation effect, where limited structural disruption occurred, insufficient to unlock the full bioavailability of the organic matter.

### Comparative analysis with existing literature on pretreatment and anaerobic digestion

The obtained findings align with existing literature demonstrating the efficacy of pretreatment methods in enhancing anaerobic digestion by improving substrate biodegradability and microbial activity (Karthikeyan et al., 2024).

The observed mechanism of UV light breaking down complex organic structures is consistent with previous research; for example, a UV-driven photocatalytic technique increased methane yield in wheat straw by 57% due to significant lignin degradation and improved solubilisation. (Muhammad Awais et al., 2020). Similarly, UV irradiation combined with a  $\text{TiO}_2$  photocatalyst led to a 37% increase in methane yield in biochemical methane potential assays (Alvarado-Morales et al., 2017) supporting the idea that microorganisms

can efficiently utilise UV-induced structural breakdown products.

The reduction in COD removal and methane yield at excessive UV exposures is also consistent with the concept that over-exposure can lead to the formation of undesirable intermediates that inhibit microbial activity (Karthikeyan et al., 2025; Ran and Li, 2020). Although a direct link between prolonged UV pretreatment and the formation of specific methanogenesis inhibitors like furans or phenolics is not yet established by direct studies, analogies from hydrothermal and other photodegradative systems suggest that excessive photolysis can generate intermediate compounds (e.g., 5-hydroxymethylfurfural, furfural, phenolic derivatives) known to inhibit methanogenic activity by damaging microbial cell membranes or inhibiting enzymatic pathways. Furthermore, the importance of particle size reduction through pretreatment, as suggested by the obtained findings, is supported by studies showing enhanced structural removal and enzymatic hydrolysis efficiency with reduced particle size. (Yang et al., 2023). The inherent limitations of untreated cow dung, specifically its complex lignocellulosic matrix, which restricts microbial access, are well-documented, highlighting the critical role of pretreatment in enhancing anaerobic digestibility (Orlando and Borja, 2020).

### Limitations and future research directions

While this study offers valuable insights into the effect of UV-light pretreatment on cow dung anaerobic digestion, it has certain limitations. A major limitation is that the potential inhibitory compounds that may have affected digestion performance were not measured. Cow dung can contain inherent inhibitors such as phenolic derivatives, tannins, and long-chain fatty acids, which are known to suppress microbial activity. Additionally, prolonged UV irradiation ( $\geq 150$  min) may have produced secondary inhibitory by-products through photolytic degradation of organic matter. Such compounds could include furans (e.g., furfural and 5-hydroxymethylfurfural), phenolics, and low-molecular-weight aromatics, which have been reported to interfere with enzymatic activity and disrupt methanogenesis (Ran and Li, 2020; Karthikeyan et al., 2025). The observed reduction in methane yield and COD removal under extended UV exposure in the conducted study aligns with these

inhibitory effects. Future research should therefore employ advanced analytical techniques such as HPLC, GC-MS, or LC-MS to identify and quantify specific inhibitory intermediates, alongside microbial community profiling, to better understand the mechanistic links between UV pretreatment, inhibitor formation, and anaerobic digestion performance.

Another limitation is that microbial community analysis was not conducted, which restricts the ability to correlate shifts in microbial consortia with the observed changes in methane yield and COD removal efficiency under different UV exposures. Microbial dynamics are critical in determining the resilience and adaptability of methanogenic communities to potential inhibitors, and future studies should incorporate molecular tools such as 16S rRNA sequencing or metagenomics to bridge this gap.

Furthermore, this work was conducted under controlled laboratory-scale conditions. While the results demonstrate the potential of UV-light pretreatment to improve substrate digestibility, the feasibility of scaling up this approach and its energy balance must be critically evaluated under pilot- and field-scale settings. Cost-benefit analyses, combined with life-cycle assessments, will be essential to determine whether UV pretreatment is a sustainable option compared to other existing methods.

### Implications for sustainable biogas production and waste-to-energy processes

The obtained findings suggest that UV-light pretreatment is a promising method for enhancing anaerobic digestion of cow dung, but its application requires careful optimisation of exposure duration. The identification of optimal UV exposure times (90 and 120 minutes) is crucial for maximising methane production and COD removal efficiency. These results underscore the importance of balancing the enhancement of substrate accessibility with the prevention of inhibitory compound formation.

It is also essential to consider the energy efficiency of UV pretreatment. At the laboratory scale, the energy input for 120 minutes of exposure using a 60 W UV lamp is approximately 0.12 kWh per treatment. The improvement in methane yield at this condition was  $\sim 70$  mL/day above the control, which over 35 days corresponds to  $\sim 2.45$  L CH<sub>4</sub>. Using a conversion factor of 10 kWh/

$\text{m}^3 \text{CH}_4$ , this represents an energy recovery of  $\sim 0.0245 \text{ kWh}$ . Thus, under the conditions tested, the energy input exceeded the energy recovered, indicating that the process is not yet energy-efficient. Similar challenges, such as ultrasonic disintegration, where energy demand may outweigh the biogas gains if not optimised, have been reported in related pretreatments (Paul et al., 2023; Witaszek et al., 2020).

Nevertheless, these results should be interpreted as proof-of-concept rather than a techno-economic validation. Advances in UV-LED technology with higher energy efficiency (MacIsaac et al., 2023), integration with renewable energy sources, and application to high-solid feedstocks could reduce energy input requirements and improve the overall energy balance. Comparable studies using UV-assisted photocatalytic systems have demonstrated significant improvements in methane yield from lignocellulosic biomass (Alvarado-Morales et al., 2017; Muhammad Awais et al., 2020). Therefore, it is recommended that future research incorporate comprehensive energy balance calculations, life-cycle assessment, and techno-economic analyses to determine the feasibility of scaling UV pretreatment in sustainable biogas production systems.

## CONCLUSIONS

This study demonstrated that UV light pretreatment, when applied at an optimal duration, can enhance the anaerobic digestion of cow dung. An exposure time of 120 minutes emerged as the most effective, improving both methane yield and COD removal, while excessive exposure, particularly at 180 minutes, diminished performance. These outcomes highlight the importance of defining and maintaining an optimal UV threshold to overcome hydrolysis limitations and maximise both biogas production and organic matter removal. However, the possibility that prolonged UV exposure generates inhibitory by-products such as furans or phenolic compounds, which may suppress microbial activity, should not be overlooked. UV light pretreatment, therefore, represents a promising strategy for improving the efficiency of waste-to-energy processes. Future work should assess microbial community responses, identify inhibitory intermediates, and evaluate scale-up and economic feasibility to better inform practical application.

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