

## Effects of pretreatment strategies on hydraulic retention time of dairy cow manure through anaerobic digestion – exploratory study

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### ABSTRACT

Conventional dairy farming in Poland is associated with environmental challenges, particularly related to inadequate manure management, which may lead to water, soil, and air pollution. Dairy cow manure, typically composed of animal excreta and wheat straw, represents a valuable resource for anaerobic digestion, enabling the production of renewable energy (biogas recovering) and stabilized digestate suitable for soil fertilization/amendment while mitigating greenhouse gas emissions. However, the high lignocellulosic content present at cow manure limits the biodegradability by hindering the hydrolysis stage, thereby reducing process efficiency and prolonging hydraulic retention time. Pretreatment strategies can increase substrate accessibility by promoting the release of fermentable compounds. This study evaluates the effects of mechanical (particle size reduction) and thermal (pasteurization at 70 °C for one-hour) pretreatments on hydraulic retention time, biogas yield, and methane production during anaerobic digestion of cow manure. Biomethane potential tests were conducted in accordance with DIN 4630:2016 standards. The results demonstrated that all pretreatment strategies increased digestion kinetics, leading to shorter fermentation times. The most significant reduction was observed for pasteurized fresh manure, achieving up to 46% reduction in hydraulic retention time to reach 80% of cumulative methane production, although accompanied by a decrease in methane yield (-8.7%). Mechanical pretreatment showed more balanced performance, with the 5–10 mm particle size achieving the highest biogas ( $468.0 \pm 86.0$  m<sup>3</sup>/Mg OM) and methane yields ( $265.5 \pm 36.0$  m<sup>3</sup>/Mg OM), corresponding to increases of 11% and 6.9%, respectively, compared to untreated cow manure. Generally, the results showed that while pretreatments accelerate biodegradation, optimizing anaerobic digestion performance demands balancing hydraulic retention time reduction and methane production. This exploratory study gives relevant information for improving manure treatment and management and increasing the efficiency of anaerobic digestion units.

**Keywords:** renewable energy; biogas; milling; pasteurization; biomethane potential test, retention time.

### INTRODUCTION

The dairy sector plays a key role in the global food system, driven by increasing demand for milk and dairy products (Organization for Economic Cooperation and Development [OECD]/ Food and Agriculture Organization [FAO]

– OECD/FAO, 2023; Vinci, 2024). Worldwide, productivity gains are expected to result more from improved efficiency than from herd expansion (Clay et al., 2020). As milk production expands, so do the associated environmental pressures of dairy farming, including Greenhouse gases (GHG) emissions, soil and water pollution,

nutrient (mainly nitrogen and phosphorus) imbalances, pathogen proliferation, and the increasing production of biowaste alongside inefficient dairy cow manure (CM) management systems (Cárdenas et al., 2021; Li et al., 2021; Shahbandeh, 2023; Vinci, 2024). Hence, in the coming years, dairy farming in the European Union is expected to face significant challenges, mainly due to the implementation of stricter environmental regulations regarding biowaste management and fluctuations in the milk prices (De Otálora et al., 2024; Vingerhoets et al., 2025). Consequentially, several studies have investigated strategies to increase farm resilience to environmental, social, and economic pressures (Bhat et al., 2022; Clay et al., 2020; Petersen et al., 2007).

Despite years of research, no consensus has been reached regarding the most sustainable dairy farming system, as both intensive and extensive systems present trade-offs in environmental performance (De Otálora et al., 2021; Latruffe et al., 2023; Moscovici Joubran et al., 2021). Among the various environmental challenges, carbon dioxide (CO<sub>2</sub>), nitrous oxides compounds (NO<sub>x</sub>), and methane (CH<sub>4</sub>), mainly from enteric fermentation and manure storage, represent major contributors to the sector's climate impact (Britt et al., 2021). In pasture-based system, more than 50% of total GHG emissions are associated with CH<sub>4</sub> from enteric fermentation, whereas in other regions (especially from Europe and North America), a significant share comes from manure management, feed production, and fertilizer use (Mazzetto et al., 2022). While dietary interventions can mitigate enteric CH<sub>4</sub> emissions, improving CM management remains a critical intervention point (Gao et al., 2025).

In Europe, CM management is mainly addressed by a traditional dairy cow management systems approach, involving long-term storage (often several months) in piles, followed by land application during appropriate agronomic periods (e.g., before sowing or after harvest). Such long-term manure storage can significantly impact on the environment through emissions of GHG, such as CH<sub>4</sub>, nitrous oxide (N<sub>2</sub>O) as well as odorous gases such as ammonia (NH<sub>3</sub>) and hydrogen sulfide (H<sub>2</sub>S). Besides, nutrient leaching may occur, potentially contaminating soil, groundwater, and surface water (Kadam et al., 2024; Symeon et al., 2025; Vingerhoets et al., 2025).

In this context, AD of CM emerges as a promising alternative to boost sustainability in dairy

farming by enabling more efficient biowaste management while providing environmental and economic benefits. These include resource recycling and the generation of value-added products such as biogas and digestate (European Biogas Association [EBA], 2025; Kim et al., 2023; Kunz et al., 2019). Anaerobic digestion is a biological process occurring in the absence of oxygen, in which organic matter is degraded by microorganisms in controlled reactors. This process reduces GHG emission while producing both renewable energy in the form of biogas and digestate, a stabilized organic by-product potentially suitable for soil amendment (Gugliucci et al., 2024; Kunz et al., 2019; Pulka & Dach, 2022; Scott & Blanchard, 2021).

The HRT is a key parameter for AD performance and refers to the duration that the feedstock remains within the reactor. A longer generally enhances the degree of sludge stabilization, leading to a more stable digestion process. It also promotes effective contact between biomass and the liquid phase, thereby improving substrate degradation and increasing biogas yields. To achieve the optimum performance of mesophilic microorganisms, the hydraulic retention time (HRT) typically ranges from 10 to 40 days (Kothari et al., 2014). Nonetheless, shorter HRT are associated with lower capital and operational costs, making them economically attractive. Reducing HRT while maintaining fermentation efficiency is therefore desirable, as it can improve overall process economics (Hidalgo-Sánchez et al., 2023). Pretreatment strategies applied prior to fermentation can enhance AD performance by accelerating hydrolysis, increasing biogas yield, reducing HRT, and improving digestate stability. These strategies aim to break down complex organic structures and increase substrate accessibility to hydrolytic enzymes, thereby improving overall process efficiency (Anacleto et al., 2022; Kupryaniuk et al., 2020; Mankar et al., 2021; Nauman Aftab et al., 2019).

More than 70% of biogas plants in Europe operate on agricultural substrates such as CM. (Noori et al., 2021). However, these systems often present limited energy efficiency due to the high content of lignocellulosic material (lignin, cellulose, and hemicellulose), which is resistant to microbial degradation. Lignocellulose typically accounts for 40–60% of CM dry matter (Li et al., 2021; Noori et al., 2021). This leads to slow hydrolysis, incomplete digestion, suboptimal

biogas yields, less stabilized digestate, and/or longer HRT (Dach et al., 2020; Li et al., 2021).

Mechanical, thermal, chemical, and biological pretreatment strategies have demonstrated varying degrees of success in enhancing CH<sub>4</sub> production. Mechanical, thermal, and biological pretreatments have shown moderate improvements in biogas production at the laboratory scale and are applicable to full-scale systems (Anacleto et al., 2022; Baksi et al., 2023). Furthermore, meta-analyses indicate that mechanical and chemical pretreatments are particularly effective for herbivore manure, including CM, while biological approaches tend to yield more modest improvements (Anacleto et al., 2022; Chen et al., 2017; Mankar et al., 2021; Orlando & Borja, 2020; Prasad et al., 2022).

Despite the substantial body of research focused on enhancing biogas yield through pretreatment strategies, relatively few studies have evaluated their effects on HRT. Hydraulic Retention Time is a critical operational parameter in AD systems. While increasing the HRT can improve substrate degradation, it also leads to higher operational costs. Besides, the HRT strongly influences microbial growth dynamics; insufficient retention time may result in biomass washout and eventual process failure (Kim et al., 2023). Therefore, the present study address research gap by investigating the effects of mechanical and thermal pretreatments on the HRT of AD systems fed with CM. The study evaluates three mechanical pretreatment strategies to reduce the size of the substrates (shredding, milling, and deep milling) and one thermal (pasteurization), assessing performance indicators including biogas yield, methane yield, biogas production rate, biomethane production rate, and gas composition (CH<sub>4</sub>, O<sub>2</sub>, CO<sub>2</sub>, H<sub>2</sub>S, and NH<sub>3</sub>).

## MATERIALS AND METHODS

### Sample preparation

The CM was obtained from the Przybroda Experimental Farm of Poznań University of Life Sciences (PULS), Poznań, Poland. The Przybroda Farm is one of nine PULS experimental units and has operated a modern agricultural biogas plant since 2019, with an installed electrical capacity of 0.499 MW. The biogas plant processes various types of biowaste from agri-food industries and

livestock production. Since 2022, the heat generated by the Combined Heat and Power (CHP) unit has supplied approximately one-third of the energy required by the local community of Przybroda.

The collected CM consisted of dairy cow excreta mixed with wheat straw used as bedding material. The material was obtained immediately after morning manure removal during routine barn-cleaning at the Przybroda Farm. Approximately 100 kg of CM was collected and transported to the Ecotechnologies Laboratory at the Department of Biosystems, PULS. After thorough homogenization by mechanical mixing, standardized subsamples were prepared for subsequent analysis. One of the subsamples was dried in two phases: (i) outdoor drying, during which the material was manually aerated twice daily for nine days at ambient temperatures ranging between 9 to 22 °C; (ii) controlled indoor drying, carried out in a cool, dry room at 25 °C for an additional 19 days.

Another subsample intended for physicochemical analyses was stored under refrigeration at 4 °C.

### Physical and chemical characterization

Physicochemical analyses of both the substrate (feedstock) and the inoculum (INC) were conducted in triplicate, accordance with Polish standards for dry matter (DM), organic matter (OM), pH and electrical conductivity (EC). These measurements were used to define appropriate substrate-to-inoculum ratios and to express biogas and CH<sub>4</sub> yields expressed per Mg of OM (% of DM). The dry matter was determined according to the EN ISO 18134-3:2015 standard. The substrate samples were dried in a SUP-18G drying oven at 105±5 °C until a constant weight was achieved. The organic dry matter of the substrate was determined according to the BS EN 12879:2000 standard. The substrate samples were burnt in a Lenton AF 11/6b muffle furnace at 550±25 °C until a constant weight was achieved. The EC and pH value was measured with the potentiometric method according to the BS EN 12879:2000 standard. The pH value in the substrate samples was measured with an Elmetron CP-411 meter with an ERH-111 combination electrode and a temperature sensor with a Pt-1000B resistor to compensate the pH value depending on the temperature of the sample.

### Pretreatments strategies

Mechanical and thermal pretreatment strategies were applied to homogenized CM. One of the subsamples was separated and subjected to the two-stage drying process (outdoor followed by controlled indoor drying), hereafter referred to as dried cow manure (CMDM), which was subsequently used for mechanical pretreatments. The other subsample was manually cut into approximately 13 mm pieces and subjected to the thermal pretreatment, as illustrated in Figure 1.

**Mechanical pretreatment:** to study the influence of particle-size on the performance of this type of pretreatment on the retention time of AD process of CM, different size ranges were assessed. The pretreatment applied to CMDM was performed in three sequential stages: (1) shredding: the substrate was shredded using a hammer mill H-111/3 (Jaworzno, Poland) equipped with an 8 mm perforated screen, producing particles size of 5-10 mm [dry dairy cow manure 5-10 mm (CM5)]; (2) milling: a portion of the CM5 material was further milled, producing particles size of 1–2 mm [dry dairy cow manure 1–2 mm (CMS)]; and (3) deep milling: final size reduction from CMS was achieved using a Cutting Mill SM 200 (RETSCH GmbH, Germany), producing a powder-like material with particle sizes less than 0.5 mm [dry dairy cow manure 0.5 mm (CMP)].

**Thermal pretreatment:** in accordance with the European Union regulation (EC) no. 1069/2009, animal byproducts must be subjected

to pasteurization (70 °C for one-hour) prior to AD to reduce microbial risks. Thermal pretreatment was therefore applied both as a hygienization step and as a potential method to enhance biodegradability (Yin et al., 2020). The methodology was adapted from Michailidou et al. (2024), Nolan et al. (2018), and Yin et al. (2020), and applied to both fresh CM (after homogenization and cutting to approximately 13 mm) and CMS samples. A dilution ratio of 1:10 (w/v) was applied prior to pretreatment. The samples were placed in sealed glass containers to prevent evaporation and treated in an SLW 32 STD oven (POL-EKO-APPARATURA, Poland) at 70±1 °C. Once the internal temperature of the substrate reached 70 °C, the pasteurization period (one-hour) was initiated, with continuous temperature monitoring. This procedure resulted in the following samples: fresh dairy cow manure after pasteurization (FMP) and dried dairy cow manure (1-2 mm), after pasteurization (DMT). After treatment, all samples were cooled to room temperature and stored at 4 °C until BMP test.

### Biochemical methane potential test

The BMP assay was conducted according to internal laboratory procedures based on the standards DIN 38414-8:1985-06/1985 and VDI 4630/2016. The VDI 4630 guideline specifies requirements for equipment, INC characteristics, and operational conditions for assessing the biodegradability of organic substrates. The substrate/

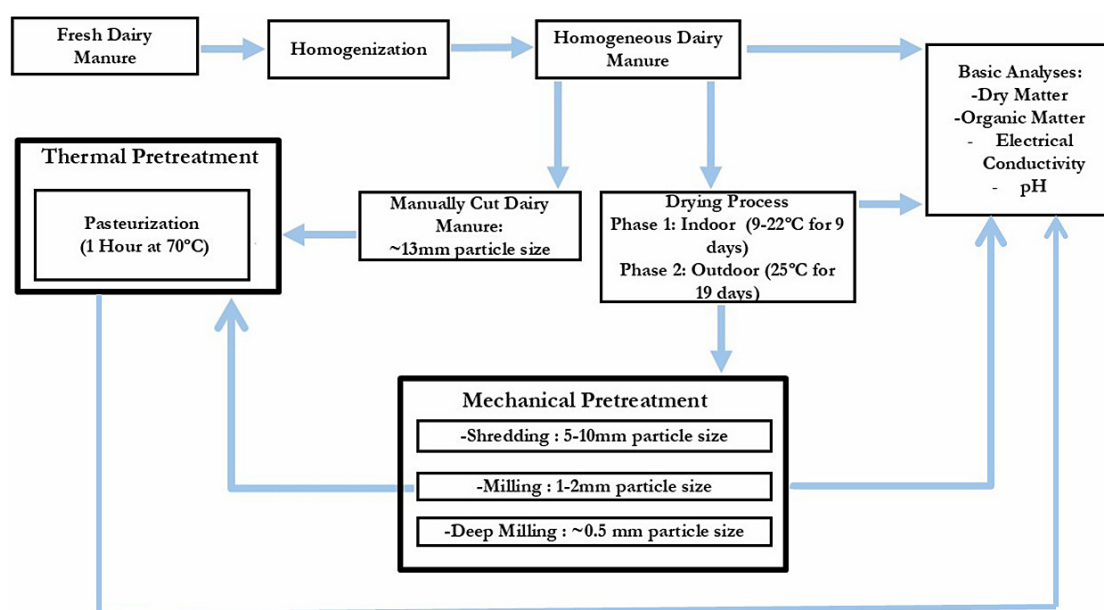


Figure 1. Scheme of the pretreatment strategies applied in the study

inoculum ratio used was 0.45 in accordance with the recommendation.

The INC consisted of the liquid fraction obtained after solid-liquid separation of digestate from mixed organic waste subjected to mesophilic AD ( $38^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) at the Ecotechnologies Laboratory. This INC was selected due to its diverse microbial community and its suitability for degrading a wide range of substrates. It was stored under anaerobic conditions  $25 \pm 1^{\circ}\text{C}$  prior to use.

The BMP system consisted of a multi-chamber batch configuration as previously described by Czekala et al. (2025) and Waliszewska et al. (2024), using reactors with 1.8 m<sup>3</sup> or working volume. The experimental setup was conducted in three phases: (i) on July 1st, six samples were prepared (INC, CM, CMDM, CM5, CMS, and CMP); (ii) on July 4th, the FMP sample was prepared; and (iii) on July 7th, the DMT sample was prepared. The assays were conducted until the HRT criterion was reached for all the three repetitions for each tested sample. According to VDI 4630/2016, the termination criterion was defined as daily biogas production corresponding to less than 1% of cumulative biogas generated. Most biogas production occurred within the first week, with biodegradation approaching completion after 20 days. After approximately 40 days, gas production was minimal. Biogas volume was recorded every 24 hours. Gas composition (CH<sub>4</sub>; CO<sub>2</sub>; NH<sub>3</sub>, H<sub>2</sub>S, and O<sub>2</sub>) was measured using a GA5000 certified gas analyzer with an accuracy of 0.01 dm<sup>3</sup> (Geotech's, UK).

In this exploratory study, as referred before, HRT was operationally defined based on batch digestion performance, in accordance with VDI4630/2016 guidelines. Since BMP assays are conducted under batch conditions without continuous inflow or outflow, HRT does not correspond to its classical engineering definition (i.e., reactor volume divided by influent flow rate). Instead, HRT was interpreted as the effective fermentation time required to achieve near-complete substrate degradation. Specifically, HRT was calculated as the total number of days required until daily CH<sub>4</sub> production fell below 1% of the cumulative CH<sub>4</sub> production, which is the standard termination criterion for BMP test according to VDI 4630/2016. This approach used on this study enables the estimation of the expected HRT under practical conditions and allows the comparison of the impact of different pretreatment strategies on fermentation kinetics and process efficiency. The “effective HRT” defined here should not be

directly interpreted as representative of full-scale or continuous reactor operation, as this may lead to an overestimation of its practical applicability. Briefly, BMP tests provide information regarding: the fundamental biogas yield and anaerobic biodegradability of a material or mixture; the qualitative rate of anaerobic degradation; the qualitative identification of inhibitory effects within the tested concentration range. Nevertheless, BMP tests do not provide information regarding: process stability under continuous feeding conditions; biogas yields under practical operating regimes where synergistic effects may occur; the mono-digestibility of substrates under process conditions, and operational limits such as maximum organic loading rates (VDI 4630/2016). Additionally, key phenomena such as microbial washout community adaptation (Kappell et al., 2020), accumulation of intermediates (e.g., volatile fatty acids) (Beschkov & Angelov, 2025), and mass transfer limitations are not captured in batch systems (Sponza & Öztekin, 2023).

### Statistical analyses

The data was analyzed using descriptive statistics in Excel® software. Mean values, standard deviations, standard errors, and minimum and maximum values were calculated for all relevant parameters. All samples were tested in triplicate. Outliers and anomalous values were carefully evaluated and, when justified, excluded prior to the determination of the final mean values to ensure data reliability and robustness. Additionally, an analysis of variance (ANOVA) was performed to evaluate differences among groups, under 95% confidence interval.

## RESULTS AND DISCUSSION

### Physicochemical characterization of substrate samples

The physicochemical parameters of the INC and substrate samples used in the BMP assays are presented in Table 1. The INC exhibited the lowest values of EC, DM, and OM among all analyzed samples, which is consistent with VDI 4630/2016 requirements for BMP assays. According to this guideline, the INC should contain more than 50% OM relative to DM to ensure sufficient microbial activity. The measured pH (7.40) was within the

optimal neutral range for AD, in agreement with values reported in literature (Kafle & Chen, 2016; Kozłowski et al., 2019). These characteristics confirm the suitability of the INC for supporting stable digestion and reliable CH<sub>4</sub> production.

Marked differences in physicochemical parameters were observed among the substrate samples because of the applied pretreatment strategies. The pH values varied considerably, reflecting changes in biochemical conditions that directly influence microbial activity and digestion kinetics. Thermally pretreated samples (DMT and FMP) exhibited near-neutral pH values, whereas untreated and mechanically pretreated samples showed alkaline conditions (Table 1).

This shift toward neutrality after thermal pretreatment can be attributed to partial inactivation of hydrolytic microorganisms and the consequent reduction in NH<sub>3</sub> formation. In contrast, untreated manure tends to become alkaline due to microbial conversion of nitrogen compounds into NH<sub>3</sub>, which increases pH. Since pH is a critical parameter affecting methanogenic activity, these differences are expected to influence both the rate of CH<sub>4</sub> production and the effective retention time required for process stabilization (Li et al., 2021).

Dry matter content showed the most pronounced variation among samples (p<0.05) and represents a key factor influencing AD performance. Fresh CM exhibited the lowest DM content (22.00%), whereas the two-stage drying process increased DM substantially CMDM (92.93%) as previously stated and reported in literature (Coarita Fernandez et al., 2020; Kunz et al., 2019; Schumacher et al., 2020). Similar

high DM values were observed in mechanically pretreated samples (CM5, CMS, and CMP), indicating that particle size reduction did not change the solids content. Thermally pretreated samples (DMT and FMP) exhibited very low DM values (approximately 1.7%), due to the mandatory dilution step (1:10 w/v) applied prior to pasteurization, as also referred by Samadamaeng et al. (2024). These differences in DM content are very highly relevant for AD kinetics, as high-solids substrates may limit mass transfer, microbial activity, and enzyme diffusion, thereby slowing the first phase of the degradation process (hydrolysis) and prolonging HRT. Conversely, diluted substances enhance substrate-microorganism contact, which may accelerate biodegradation and reduce the effective retention time (Kunz et al., 2019; Schumacher et al., 2020).

Organic matter content, expressed on a DM basis, remained relatively stable amongst the samples however presented considerable variance among groups (p<0.05), with values close to 90% for CM and pretreated substrate samples. Slight reductions were determined in dried samples, being attributed to partial aerobic degradation of substrates during drying process as referred by De Otálora et al. (2021). Prominently, the absence of major deviations in OM content indicates that the pretreatment strategies primarily affected the physical structure and availability of organic compounds rather than their overall quantity. The results suggested that differences in digestion performance and HRT are more strongly associated with the changes in the substrate’s accessibility than compositional alterations.

**Table 1.** Physicochemical characterization of the inoculum and substrate samples

Substrate samples <sup>†</sup>	Pretreatment <sup>†</sup>	Physicochemical parameters <sup>‡</sup> (mean ± standard deviation)			
		pH	EC [mS/cm]	DM [%]	OM [%DM]
INC	N/A	7.40	14.00 ± 8.00	2.86 ± 0.03	53.76 ± 0.00
CM	N/A	8.42	30.69 ± 5.67	22.00 ± 1.24	90.25 ± 1.03
CMDM		9.40	112.63 ± 0.57	92.93 ± 0.58	89.46 ± 0.22
CM5	Mechanical	9.47	96.49 ± 0.28	93.23 ± 0.10	89.08 ± 0.93
CMS		9.41	104.03 ± 1.70	93.37 ± 0.02	87.66 ± 0.22
CMP		9.40	96.36 ± 0.78	91.54 ± 1.52	88.08 ± 0.48
DMT	Thermal	7.60	4.11 ± 0.22	1.77 ± 0.02	89.64 ± 0.24
FMP		7.38	5.76 ± 2.09	1.70 ± 0.20	89.42 ± 0.91

**Note:** \* N/A – not applicable. <sup>†</sup>INC – inoculum; CM – dairy cow manure; CMDM – dried cow manure; CM5 – dry dairy cow manure 5-10 mm; CMS – dry dairy cow manure 1–2 mm; CMP – dry dairy cow manure 0.5 mm; DMT – dry dairy cow manure 1-2 mm + pasteurization; FMP – fresh dairy cow manure after pasteurization.

<sup>‡</sup>EC – electrical conductivity; DM – dry matter; OM – organic matter.

Electrical conductivity also varied significantly ( $p < 0.05$ ). The highest EC values were observed in dried and mechanically pretreated samples (up to 112.63 mS/cm for CMDM) and the lowest values in thermally pretreated samples (4.11–5.76 mS/cm). This reduction in EC is directly associated with the dilution applied prior to thermal pretreatment, which decreases ionic strength. Although EC is not a primary control parameter in AD, it reflects the concentration of soluble ions and indirectly indicates substrate concentration and moisture content. Therefore, the lower EC values observed in thermally pretreated samples further support improved conditions for mass transfer and microbial accessibility, potentially contributing to faster digestion rates. The results observed are consistent with previous studies reported in literature (Ariunbaatar et al., 2014; Carrère et al., 2010).

Generally, the results demonstrate that the applied pretreatment strategies modified key physicochemical properties of the substrate, particularly DM, content pH, and EC. These parameters are directly linked to the efficiency of the first stage of fermentation process (hydrolysis) and microbial activity and therefore play a crucial role in determining digestion kinetics and effective HRT.

It is imperative to acknowledge that the isolated impact of particle size and dried condition of the dairy cow manure sample, on hydraulic retention time, while significant, was not the focus of this study. The present study sought to evaluate the combined effects under conditions representative of the investigated process. It is important to mention that the drying process increased substrate recalcitrance and is expected to prolong

HRT, whereas thermal pretreatment combined with dilution improved substrate accessibility and may contribute to shorter retention times. Mechanical pretreatment primarily affects particle size, enhancing surface area but showing limited impact on intrinsic physicochemical composition.

### Hydraulic retention time

The retention time required to achieve 80%, 90%, and 100% of the total  $\text{CH}_4$  production is displayed in Figure 2. The results clearly demonstrate the positive effect of the applied pretreatment strategies in reducing the time required for substrate samples to complete the fermentation process. In practical terms,  $\text{CH}_4$  production was achieved at shorter retention time, indicating that pretreatments accelerate biochemical reactions within the system. This observation is very important from a practical perspective, since its shortening potentially allows processing higher amount of substrate within the same timeframe while maintaining a constant fermentation chamber volume.

The shortest HRT was observed for the FMP sample, while the longest was recorded for the CMDM sample, which underwent drying but no additional pretreatment. Complete  $\text{CH}_4$  production was achieved in 19 days for FMP, whereas CMDM required 41 days. Similar findings were reported by Michailidou et al. (2024), who observed reduced fermentation times for pasteurized substrates along 1.3-fold increase in biogas production.

As shown in Figure 2, the FMP sample required the shortest time (15 days) to reach 80% of

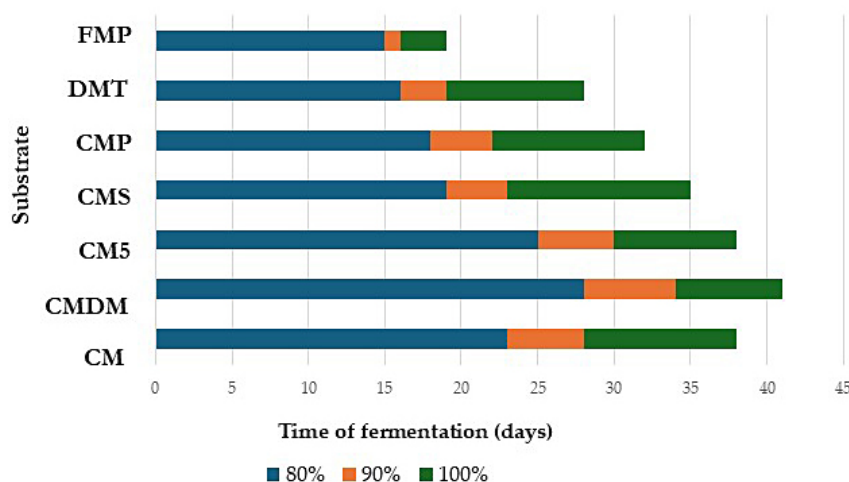


Figure 2. Time required for 80, 90, and 100% of total methane production

cumulative  $\text{CH}_4$  production. In contrast, the untreated CM sample required 28 days, corresponding to an approximate 46% reduction in HRT due to pasteurization. For mechanically pretreated samples, a progressive reduction in HRT was observed with decreasing particle size. Retention times of 41, 38, 35, and 32 days were recorded for CMDM, CM5, CMS, and CMP, respectively. Compared to the untreated CM sample, reductions of 0%, 8%, and 16% were observed for CM5, CMS, and CMP, respectively, to reach 80% of  $\text{CH}_4$  production. These results demonstrate that shorter HRT can be achieved through the application of pretreatment strategies.

To reach 100% of the  $\text{CH}_4$  production, the CM samples required 38 days, which is consistent with values reported in literature (Nleya et al., 2025). The lowest HRT was observed for the FMP sample, which stabilized after approximately 20 days. This difference highlights the strong effect of pasteurization combined with the mandatory dilution step ratio 1:10 (w/v), which increases substrate accessibility. Thermal pretreatment promotes cell lysis and the disruption of structural polysaccharides, releasing readily biodegradable compounds. This is reflected in the steeper initial slope of the FMP curve and is consistent with findings reported by Samadamaeng et al. (2024). The applied temperature (70 °C for one-hour) This abrupt gradient (70°C, one-hour) accelerated the hydrolytic phase, supporting the hypothesis that thermal pretreatment promotes enzyme-substrate interactions and accelerates early-stage AD.

## Biogas production efficiency test

The cumulative biogas production results are presented in Figure 3. All samples (except INC) exhibited sigmoidal cumulative biogas production curve, typical of AD processes, including lag (slow phase), exponential (rapid phase), and plateau phases as reported in literature Kozłowski et al., 2019). BMP test presented results consistent with kinetic behavior commonly detected for this samples under mesophilic conditions (Kafle & Chen, 2016; Yang et al., 2017). No statistically significant differences were observed among the samples ( $p > 0.05$ ).

The CMDM sample presented the slowest biogas production rate, characterized by a prolonged lag phase due to low moisture content and structural rigidity. These factors limit microbial activity and enzymatic diffusion, which are critical for hydrolysis (De Otálora et al., 2021; Schumacher et al., 2020).

Mechanical pretreatment improved biogas production, particularly for the CMP samples, with 0.5 mm particle size, due to increased available surface area reduced diffusion limitations, which are very well recognized effects of this type of pretreatment in the disintegration on lignocellulosic biowastes (Coarita Fernandez et al., 2020; Prasad et al., 2022). Likewise, the DMT sample showed improved performance compared to untreated CM, indicating that thermal pretreatment strategy partially compensated for structured limitations.

Overall, the effectiveness of pretreatment strategy depended strongly on substrate properties,

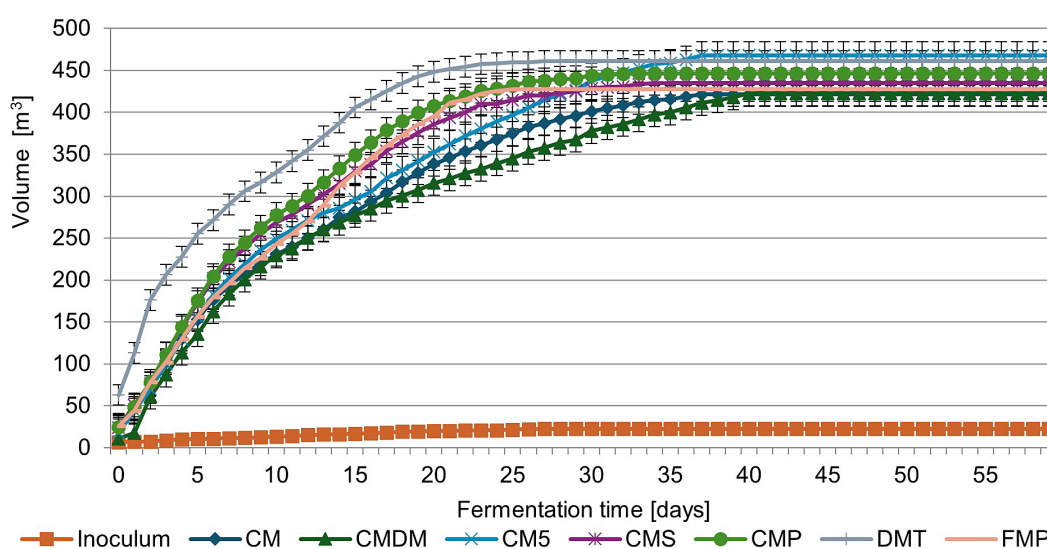


Figure 3. Cumulative biogas production ( $\text{m}^3/\text{Mg OM}$ )

particularly moisture content and structure. Small variations in the results can occur by several interacting factors: (1) high DM and OM content in the mechanical pretreatment samples, which delays mass transfer or promoted limited acid accumulation during the early stages of fermentation; (2) reduced humidity supplementation due to drying process, potentially reducing microbial access despite reduced particle size; (3) partial thermal degradation of easily digestible fractions during drying process, leaving more recalcitrant substrate in reactors with limited improvement achievable by milling alone.

### Methane production efficiency test

Cumulative  $\text{CH}_4$  production results are shown in Figure 4. All samples exhibited (except the INC), sigmoidal cumulative  $\text{CH}_4$  production curves typical of manure-based substrates under mesophilic conditions (Kafle & Chen, 2016; Kozłowski et al., 2019; Yang et al., 2017).

The FMP sample showed the lowest  $\text{CH}_4$  yield per Mg of OM, suggesting suboptimal conditions for methanogenesis, potentially due to instability or inhibitory effects. In fact, methanogenic archaea are very sensitive and strongly dependent on upstream microbial activity, and this dependency was not evidently adequately met for this substrate over AD process (Kunz et al., 2019).

The relatively poor performance of FMP substrate samples in the present study may be associated with the formation of inhibitory compounds, particularly  $\text{NH}_3$ , which are common in studied type of manure (dairy and cattle) and can

reduce the efficiency of methanogenic pathways (Nleya et al., 2025). Due to methodological limitations,  $\text{NH}_3\text{-N}$  dynamics could not be analytically assessed, preventing further interpretation of potential inhibition effects. Regardless of the performance, an analysis of variance indicated no statistically significant differences among groups ( $p > 0.05$ ).

In contrast, CM5 achieved the highest peak in cumulative  $\text{CH}_4$  yield, indicating that moderate particle size reduction provides optimal conditions for microbial activity since ensured stable availability of fermentable compounds, increased surface area and promoted continuous metabolite supply for methanogenesis (Li et al., 2021) (Witaszek et al., 2022).

The results suggested that both moisture content and particle-size reduction influence  $\text{CH}_4$  production, although their effects are not strictly proportional.

### Bioenergy production efficiency test results according to German DIN 38.414/1985

The fermentation efficiency results, including cumulative biogas and  $\text{CH}_4$  yield,  $\text{CH}_4$  content, and the variation in  $\text{CH}_4$  production relative to the untreated CM sample are presented in Table 2.

The CM sample produced  $421.2 \pm 29.0 \text{ m}^3$  biogas/Mg OM, consistent with literature values (Kozłowski et al., 2019), who obtained  $477 \pm 25 \text{ m}^3$  biogas/Mg OM in BMP tests for untreated cow manure. Among all samples, CM5 exhibited the highest biogas and  $\text{CH}_4$  production ( $422.5 \pm 87.0 \text{ m}^3$  biogas/Mg OM and  $265.5 \pm 36.0 \text{ CH}_4/\text{Mg OM}$ ,

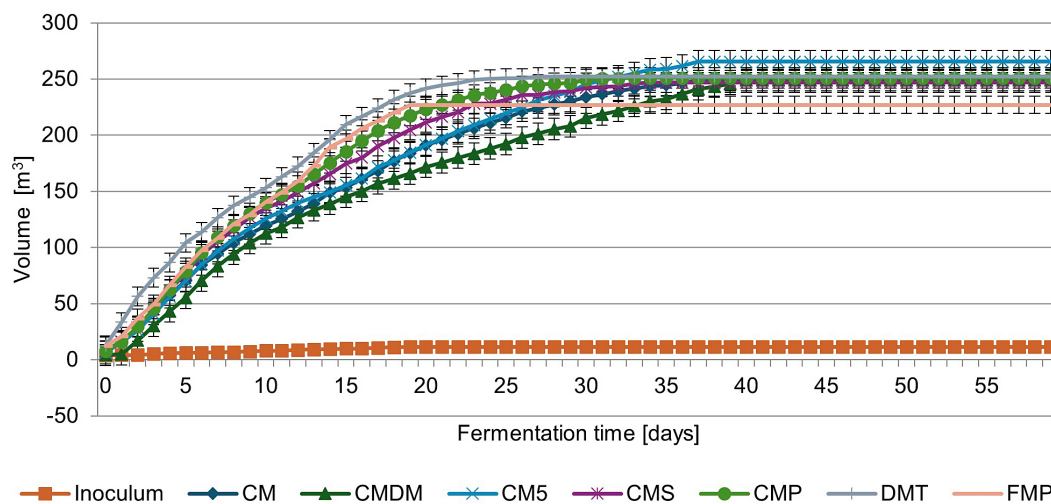


Figure 4. Cumulative methane production ( $\text{m}^3/\text{Mg OM}$ )

**Table 2.** Fermentation efficiency test results according to German DIN 38.414/1985

Substrate Samples <sup>†</sup>	Cumulated biogas [m <sup>3</sup> /Mg OM]	Changes in Biogas production [%]	CH <sub>4</sub> content [%]	Cumulated CH <sub>4</sub> [m <sup>3</sup> /Mg OM]	Changes in CH <sub>4</sub> production [%]
CM	421.2±29.0	0.0	59.0	248.6±28.0	0.0 <sup>‡</sup>
CMDM	422.5±87.0	0.3	59.0	249.2±49.0	0.2
CM5	468.0±86.0	11.1	56.7	265.5±36.0	6.8
CMS	434.7±12.0	3.2	56.8	246.8±8.0	-0.8
CMP	446.4±8.0	6.6	56.2	251.1±5.0	1.0
DMT	461.0±205.0	9.4	56.3	252.0±39.0	1.4
FMP	427.5±107.0	1.5	57.1	227.0±71.0	-8.7

**Note:** † CM – dairy cow manure; CMDM – dried cow manure; CM5 – dry dairy cow manure 5–10 mm; CMS – dry dairy cow manure 1–2 mm; CMP – dry dairy cow manure 0.5 mm; DMT – dry dairy cow manure 1–2 mm + pasteurization; FMP – fresh dairy cow manure after pasteurization.

‡ CM production is 100%.

respectively, corresponding to respectively 11.1% and 6.8% improvement in production considering CM sample's results), confirming the effectiveness of moderate mechanical pretreatment. In contrast, FMP sample showed reduced CH<sub>4</sub> production (227.0±71.0 m<sup>3</sup> CH<sub>4</sub>/Mg OM), potentially due to inhibitory compounds such as NH<sub>3</sub>, which are common in dairy and cattle manure, and can reduce the efficiency of methanogenic pathways (Nleya et al., 2025). Due to methodological limitations, NH<sub>3</sub>-N dynamics could not be analytically assessed, preventing further interpretation of potential inhibition effects.

The DMT sample performed better than FMP, suggesting a synergistic effect of thermal and mechanical treatment (1.4% increase in CH<sub>4</sub> yield and 9.4% increase in biogas yield compared to CM). The increased availability of cellulose likely supported more efficient hydrolysis and subsequent stabilization of methanogenesis (Li et al., 2021).

Overall, results indicate that pretreatment effectiveness depends on substrate characteristics (Kozłowski et al., 2019), pretreatment intensity (Samadamaeng et al., 2024), and operational conditions (Schumacher et al., 2020).

### Implications of pretreatment strategies on hydraulic retention time reduction

The results obtained in this exploratory study clearly indicate that pretreatment strategies can influence the HRT of AD systems when the aim is to valorize and treat CM. Thermal pretreatment, particularly using pasteurization process combined with dilution, showed the most pronounced effect in reducing HRT, as evidenced

by the fast CH<sub>4</sub> production and early stabilization observed in FMP sample (Figure 4). This behavior is attributed to enhanced substrate solubilization, improved mass transfer conditions, and increased accessibility of OM to hydrolytic microbial communities. In contrast, untreated and dried samples showed prolonged HRT to digest the material, confirming that high DM content and structural rigidity limit early stage of fermentation process (hydrolysis) and delay overall process kinetics.

Mechanical pretreatments also contributed to HRT reduction although their effectiveness depends on particle size and sample conditions. Moderate size reduction (sample CM5) improved AD performance by increasing surface area and facilitating microbial access, while excessive reduction in the particles size (CMS and CMP) exhibited diminishing or even negative effects, likely due to biomass compaction and reduced porosity. Generally, the results obtained highlight that while both mechanical and thermal pretreatments can increase AD efficiency, thermal appears to be more effective in accelerating digestion kinetics and reducing HRT under mesophilic conditions tested. These results also reinforce the importance of optimizing pretreatment selection based on substrate characteristics to achieve improved process performance and economic viability in biogas and, consequently, CH<sub>4</sub> production systems.

### CONCLUSIONS

The present exploratory study evaluated the effect of mechanical and thermal pretreatment

strategies on the hydraulic retention time of anaerobic digestion of dairy cow manure. The results presented highlight both the potential and limitations associated with application of these pretreatments prior to anaerobic fermentation. The tested strategies increased the kinetics of anaerobic digestion process leading to faster biodegradation; nonetheless, these improvements were not always accompanied by increases in biogas and, consequently, CH<sub>4</sub> yield.

Thermal pretreatment, specifically pasteurization of fresh dairy cow manure, resulted in a reduction of 46% in hydraulic retention time to reach 80% of cumulative CH<sub>4</sub> production. Although this acceleration of the digestion process, a decrease in CH<sub>4</sub> production (-8.7%) was determined, indicating that faster kinetics do not necessarily translate into improved process efficiency. Mechanical pretreatment showed more balanced values, with moderate particle size (5–10 mm) providing the best overall performance in terms of both CH<sub>4</sub> yield and process stability.

Optimizing anaerobic digestion of dairy cow manure requires a careful balance between reducing the hydraulic retention time and maintaining satisfactory biogas and CH<sub>4</sub> production. This exploratory data provide practical insights into how pretreatment selection can be tailored to improve anaerobic digestion process efficiency, showing the importance of considering both the kinetics enhancement and the CH<sub>4</sub> yield when thinking and designing the real scale units.

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## REFERENCES

1. Anacleto, T. M., Oliveira, H. R., Diniz, V. L., de Oliveira, V. P., Abreu, F., & Enrich-Prast, A. (2022). Boosting manure biogas production with the application of pretreatments: A meta-analysis. *Journal of Cleaner Production*, 362, 132292. <https://doi.org/10.1016/j.jclepro.2022.132292>
2. Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., & Lens, P. N. L. (2014). Pretreatment methods to enhance anaerobic digestion of organic solid waste. In *Applied Energy* (Vol. 123, pp. 143–156). Elsevier Ltd. <https://doi.org/10.1016/j.apenergy.2014.02.035>
3. Baksi, S., Saha, D., Saha, S., Sarkar, U., Basu, D., & Kuniyal, J. C. (2023). Pre-treatment of lignocellulosic biomass: review of various physico-chemical and biological methods influencing the extent of biomass depolymerization. *International Journal of Environmental Science and Technology*, 20(12), 13895–13922. <https://doi.org/10.1007/S13762-023-04838-4/TABLES/11>
4. Beschkov, V. N., & Angelov, I. K. (2025). Volatile Fatty Acid Production vs. Methane and Hydrogen in Anaerobic Digestion. In *Fermentation* (Vol. 11). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/fermentation11040172>
5. Bhat, R., Di Pasquale, J., Bánkuti, F. I., Siqueira, T. T. da S., Shine, P., & Murphy, M. D. (2022). Global Dairy Sector: Trends, Prospects, and Challenges. *Sustainability*, 14(7), 4193. <https://doi.org/10.3390/su14074193>
6. Britt, J. H., Cushman, R. A., Dechow, C. D., Dobson, H., Humblot, P., Hutjens, M. F., Jones, G. A., Mitloehner, F. M., Ruegg, P. L., Sheldon, I. M., & Stevenson, J. S. (2021). Review: Perspective on high-performing dairy cows and herds. In *Animal* (Vol. 15). Elsevier B.V. <https://doi.org/10.1016/j.animal.2021.100298>
7. BS EN 12879:2000. Characterization of Sludges. Determination of the Loss of Ignition of Dry Mass. British Standards Institution: London, UK (2000).
8. Cárdenas, A., Ammon, C., Schumacher, B., Stinner, W., Herrmann, C., Schneider, M., Weinrich, S., Fischer, P., Amon, T., & Amon, B. (2021). Methane emissions from the storage of liquid dairy manure: Influences of season, temperature and storage duration. *Waste Management*, 121, 393–402. <https://doi.org/10.1016/j.wasman.2020.12.026>

9. Carrère, H., Dumas, C., Battimelli, A., Batstone, D. J., Delgenès, J. P., Steyer, J. P., & Ferrer, I. (2010). Pretreatment methods to improve sludge anaerobic degradability: A review. In *Journal of Hazardous Materials* (Vol. 183, pp. 1–15). <https://doi.org/10.1016/j.jhazmat.2010.06.129>
10. Chen, H., Liu, J., Chang, X., Chen, D., Xue, Y., Liu, P., Lin, H., & Han, S. (2017). A review on the pretreatment of lignocellulose for high-value chemicals. In *Fuel Processing Technology* (Vol. 160, pp. 196–206). Elsevier B.V. <https://doi.org/10.1016/j.fuproc.2016.12.007>
11. Clay, N., Garnett, T., & Lorimer, J. (2020). Dairy intensification: Drivers, impacts and alternatives. In *Ambio* (Vol. 49, pp. 35–48). Springer. <https://doi.org/10.1007/s13280-019-01177-y>
12. Coarita Fernandez, H., Teixeira Franco, R., Bayard, R., & Buffiere, P. (2020). Mechanical Pretreatments Evaluation of Cattle Manure Before Anaerobic Digestion. *Waste and Biomass Valorization*, 11(10), 5175–5184. <https://doi.org/10.1007/s12649-020-01022-4>
13. Czekala, W., Frankowski, J., Sieracka, D., Pochwatka, P., Kowalczyk-Juško, A., Witaszek, K., Dudnyk, A., Zielińska, A., Wisła-Świder, A., & Dach, J. (2025). The energy efficiency analysis of sorghum waste biomass grown in a temperate climate. *Energy*, 320. <https://doi.org/10.1016/j.energy.2025.135433>
14. Dach, J., Mazurkiewicz, J., Janczak, D., Pulka, J., Pochwatka, P., & Kowalczyk-Jusko, A. (2020). Cow Manure Anaerobic Digestion or Composting - Energetic and Economic Analysis. *Proceedings of 2020 4th International Conference on Green Energy and Applications, ICGEA 2020*, 143–147. <https://doi.org/10.1109/ICGEA49367.2020.239708>
15. De Otálora, X. D., Amon, B., Balaine, L., Dragoni, F., Estellés, F., Ragaglini, G., Kieronczyk, M., Jørgensen, G. H. M., & del Prado, A. (2024). Influence of farm diversity on nitrogen and greenhouse gas emission sources from key European dairy cattle systems: A step towards emission mitigation and nutrient circularity. *Agricultural Systems*, 216, 103902. <https://doi.org/10.1016/j.agsy.2024.103902>
16. De Otálora, X. D., Del Prado, A., Dragoni, F., Estellés, F., & Amon, B. (2021). Evaluating three-pillar sustainability modelling approaches for dairy cattle production systems. *Sustainability (Switzerland)*, 13. <https://doi.org/10.3390/su13116332>
17. DIN 38414-8:1985-06, German standard methods for the examination of water, waste water and sludge; sludge and sediments (group S); determination of the amenability to anaerobic digestion (S 8) (1985).
18. EBA. (2025). *European biomethane capacity hits 7 bcm – stronger policy support needed to sustain momentum.*
19. EN ISO 18134-3:2015. Solid Biofuels—Determination of Moisture Content—Oven Dry Method Part 3: Moisture in General Analysis Sample. CEN (European Committee for Standardisation): Brussels, Belgium, 2015 (2015).
20. Gao, M., Irawan, A., El-Sherbiny, M., Szumacher-Strabel, M., Cieślak, A., Setiawan, M. A., Jallal, H., Fusaro, I., Jayanegara, A., Yanza, Y. R., & Liu, Y. (2025). Meta-Analysis of Incorporating Glucosinolates into Diets and Their Effects on Ruminant Performance, Ruminant Fermentation, Methane Emissions, Milk Composition, and Metabolic Biochemical Attributes. *Animals*, 15, 1480. <https://doi.org/10.3390/ani15101480>
21. Gugliucci, W., Pepe, O., & Ventorino, V. (2024). Biomass conversion strategies and wastewater reuse: a deep focus on hydrothermal liquefaction as a circular economy approach. In *Chemical and Biological Technologies in Agriculture* (Vol. 11). Springer Science and Business Media Deutschland GmbH. <https://doi.org/10.1186/s40538-024-00710-w>
22. Hidalgo-Sánchez, V., Behmel, U., Hofmann, J., & Borges, M. E. (2023). Enhancing Biogas Production of Co-Digested Cattle Manure with Grass Silage from a Local Farm in Landshut, Bavaria, through Chemical and Mechanical Pre-Treatment and Its Impact on Biogas Reactor Hydraulic Retention Time. *Sustainability (Switzerland)*, 15. <https://doi.org/10.3390/su15032582>
23. Kadam, R., Jo, S., Lee, J., Khanthong, K., Jang, H., & Park, J. (2024). A Review on the Anaerobic Co-Digestion of Livestock Manures in the Context of Sustainable Waste Management. In *Energies* (Vol. 17). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/en17030546>
24. Kafle, G. K., & Chen, L. (2016). Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. *Waste Management*, 48, 492–502. <https://doi.org/10.1016/j.wasman.2015.10.021>
25. Kappell, A. D., Carey, D. E., Zitomer, D. H., & McNamara, P. J. (2020). Effect of antimicrobial wash-out from anaerobic digesters on microbial community composition. *Environmental Science: Water Research and Technology*, 6, 1658–1671. <https://doi.org/10.1039/d0ew00094a>
26. Kim, S., Lee, C., Kim, J., & Kim, J. Y. (2023). Feasibility of thermal hydrolysis pretreatment to reduce hydraulic retention time of anaerobic digestion of cattle manure. *Bioresource Technology*, 384, 129308. <https://doi.org/10.1016/j.biortech.2023.129308>
27. Kothari, R., Pandey, A. K., Kumar, S., Tyagi, V. V., & Tyagi, S. K. (2014). Different aspects of dry AD for bio-energy: An overview. *Energy*. <https://www.sciencedirect.com/science/article/pii/>

- S1364032114004638
28. Kozłowski, K., Dach, J., Lewicki, A., Malińska, K., Do Carmo, I. E. P., & Czekala, W. (2019). Potential of biogas production from animal manure in Poland. *Archives of Environmental Protection*, 45, 99–108. <https://doi.org/10.24425/aep.2019.128646>
  29. Kunz, A., Steinmetz, R. L. R., Do Amaral, A. C., Pigosso, A., Wuaden, C. R., Barros, E. C., Antes, F. G., Bortoli, M., Da Silva, M. L. B., Prá, M. C., Mezzari, M. P., & Nicoloso, R. da S. (2019). *Fundamentos da digestão anaeróbia, purificação do biogás, uso e tratamento do digestato – “Fundamentals of anaerobic digestion, biogas purification, and the use and treatment of digestate”*.
  30. Kupryaniuk, K., Oniszczyk, T., Combrzyński, M., Dach, J., & Czekala, W. (2020). Process Efficiency and Energy Consumption during the Extrusion of Lignocellulosic Materials. *IOP Conference Series: Earth and Environmental Science*, 505. <https://doi.org/10.1088/1755-1315/505/1/012040>
  31. Latruffe, L., Niedermayr, A., Desjeux, Y., Dakpo, K. H., Ayouba, K., Schaller, L., Kantelhardt, J., Jin, Y., Kilcline, K., Ryan, M., & O’Donoghue, C. (2023). Identifying and assessing intensive and extensive technologies in European dairy farming. *European Review of Agricultural Economics*, 50, 1482–1519. <https://doi.org/10.1093/erae/jbad023>
  32. Li, Y., Zhao, J., Krooneman, J., & Euverink, G. J. W. (2021). Strategies to boost anaerobic digestion performance of cow manure: Laboratory achievements and their full-scale application potential. *Science of The Total Environment*, 755, 142940. <https://doi.org/10.1016/J.SCITOTENV.2020.142940>
  33. Mankar, A. R., Pandey, A., Modak, A., & Pant, K. K. (2021). Pretreatment of lignocellulosic biomass: A review on recent advances. *Bioresource Technology*, 334, 125235. <https://doi.org/10.1016/J.BIORTECH.2021.125235>
  34. Mazzetto, A. M., Falconer, S., & Ledgard, S. (2022). Mapping the carbon footprint of milk production from cattle: A systematic review. *Journal of Dairy Science*, 105, 9713–9725. <https://doi.org/10.3168/jds.2022-22117>
  35. Michailidou, I., Grigoriadou, I., Sfetsas, T., Vlachokostas, C., Arsenos, G., & Lymperopoulos, A. (2024). Enhancing Biogas Production: An Assessment of Pasteurization Effects on Poultry, Swine, Bovine Manure and Food Waste Substrates. *Sustainability*, 16(16), 7130. <https://doi.org/10.3390/sul16167130>
  36. Moscovici Joubran, A., Pierce, K. M., Garvey, N., Shalloo, L., & O’Callaghan, T. F. (2021). Invited review: A 2020 perspective on pasture-based dairy systems and products. *Journal of Dairy Science*, 104(7), 7364–7382. <https://doi.org/10.3168/jds.2020-19776>
  37. Nauman Aftab, M., Iqbal, I., Riaz, F., Karadag, A., & Tabatabaei, M. (2019). Different Pretreatment Methods of Lignocellulosic Biomass for Use in Bio-fuel Production. In *Biomass for Bioenergy - Recent Trends and Future Challenges*. IntechOpen. <https://doi.org/10.5772/intechopen.84995>
  38. Nleya, Y., Young, B., Noorae, E., & Baroutian, S. (2025). Anaerobic digestion of dairy cow and goat manure: Comparative assessment of biodegradability and greenhouse gas mitigation. *Fuel*, 381. <https://doi.org/10.1016/j.fuel.2024.133458>
  39. Nolan, S., Waters, N. R., Brennan, F., Auer, A., Fenton, O., Richards, K., Bolton, D. J., Pritchard, L., O’Flaherty, V., & Abram, F. (2018). Toward Assessing Farm-Based Anaerobic Digestate Public Health Risks: Comparative Investigation With Slurry, Effect of Pasteurization Treatments, and Use of Miniature Bioreactors as Proxies for Pathogen Spiking Trials. *Frontiers in Sustainable Food Systems*, 2. <https://doi.org/10.3389/fsufs.2018.00041>
  40. Noori, Cata Saady; Fatemeh, Rezaeitavabe; Juan, E. R. E. (2021). Chemical Methods for Hydrolyzing Dairy Manure Fiber: A Concise Review. *Energies*. <https://doi.org/https://doi.org/10.3390/en14196159>
  41. OECD/FAO. (2023). *OECD-FAO Agricultural Outlook 2023-2032*. OECD. <https://doi.org/10.1787/08801ab7-en>
  42. Orlando, M. Q., & Borja, V. M. (2020). Pretreatment of animal manure biomass to improve biogas production: A review. In *Energies* (Vol. 13). MDPI AG. <https://doi.org/10.3390/en13143573>
  43. Petersen, S. O., Sommer, S. G., Béline, F., Burton, C., Dach, J., Dourmad, J. Y., Leip, A., Misselbrook, T., Nicholson, F., Poulsen, H. D., Provolo, G., Sørensen, P., Vinnerås, B., Weiske, A., Bernal, M. P., Böhm, R., Juhász, C., & Mihelic, R. (2007). Recycling of livestock manure in a whole-farm perspective. *Livestock Science*, 112, 180–191. <https://doi.org/10.1016/j.livsci.2007.09.001>
  44. Pochwatka, P., Kowalczyk-Juško, A., Sołowiej, P., Wawrzyniak, A., & Dach, J. (2020). Biogas plant exploitation in a middle-sized dairy farm in Poland: Energetic and economic aspects. *Energies*, 13. <https://doi.org/10.3390/en13226058>
  45. Prasad, B. R., Padhi, R. K., & Ghosh, G. (2022). A review on key pretreatment approaches for lignocellulosic biomass to produce biofuel and value-added products. *International Journal of Environmental Science and Technology* 2022 20:6, 20(6), 6929–6944. <https://doi.org/10.1007/S13762-022-04252-2>
  46. Pulka, J., & Dach, J. (2022). Fermentation as a one of the possibilities of ghg and ammonia mitigation from dairy farming. *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM*, 22, 281–286. <https://doi.org/10.5593/sgem2022V/4.2/s19.35>

47. Samadamaeng, N., Sawatdeenarunat, C., & Charnnok, B. (2024). Enhancing biogas production from cattle manure: A circular economy approach with solar thermal pretreatment and soil conditioning. *Journal of Environmental Management*, 368, 122086. <https://doi.org/10.1016/J.JENVMAN.2024.122086>
48. Schumacher, B., Zerback, T., Wedwitschka, H., Weinrich, S., Hofmann, J., & Nelles, M. (2020). The influence of pressure-swing conditioning pretreatment of cattle manure on methane production. *Bioengineering*, 7(1), 6. <https://doi.org/10.3390/bioengineering7010006>
49. Scott, A., & Blanchard, R. (2021). The role of anaerobic digestion in reducing dairy farm greenhouse gas emissions. *Sustainability (Switzerland)*, 13, 1–18. <https://doi.org/10.3390/su13052612>
50. Shahbandeh, M. (2023, August 8). *Dairy cow numbers in the European Union (EU-27) in 2022, by country*. Statista. <https://www.statista.com/statistics/616201/dairy-cow-numbers-european-union-eu/>
51. Sponza, D. T., & Öztekin, R. (2023). Heat and Mass Limitations in an Anaerobic Digestion Process. *WSEAS Transactions on Heat and Mass Transfer*, 18, 119–139. <https://doi.org/10.37394/232012.2023.18.11>
52. Symeon, G. K., Akamati, K., Dotas, V., Karatosidi, D., Bizelis, I., & Laliotis, G. P. (2025). Manure Management as a Potential Mitigation Tool to Eliminate Greenhouse Gas Emissions in Livestock Systems. In *Sustainability (Switzerland)* (Vol. 17). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/su17020586>
53. VDI 4630, Fermentation of organic materials - Characterization of the substrate, sampling, collection of material data, fermentation tests (2016).
54. Vinci, C. (2024, July). *The EU dairy sector: Main features, challenges and prospects*. European Parliament. [https://www.europarl.europa.eu/RegData/etudes/BRIE/2018/630345/EPRS\\_BRI\(2018\)630345\\_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/BRIE/2018/630345/EPRS_BRI(2018)630345_EN.pdf)
55. Vingerhoets, R., Spiller, M., Schoumans, O., Vlaeminck, S. E., Buysse, J., & Meers, E. (2025). Economic potential for nutrient recovery from manure in the European union. *Resources, Conservation and Recycling*, 215. <https://doi.org/10.1016/j.resconrec.2024.108079>
56. Waliszewska, B., Waliszewska, H., Grzelak, M., Majchrzak, L., Gawel, E., Murawski, M., Sieradzka, A., Vaskina, I., & Spek-Dźwigala, A. (2024). Evaluation of Changes in the Chemical Composition of Grasses as a Result of the Methane Fermentation Process and Biogas Production Efficiency. *Energies*, 17. <https://doi.org/10.3390/en17164100>
57. Witaszek, K., Herkowiak, M., Pilarska, A. A., & Czekala, W. (2022). Methods of Handling the Cup Plant (*Silphium perfoliatum* L.) for Energy Production. *Energies*, 15. <https://doi.org/10.3390/en15051897>
58. Yang, Q., Wang, H., Larson, R., & Runge, T. (2017). Comparative study of chemical pretreatments of dairy manure for enhanced biomethane production. *BioResources*. <https://bioresources.cnr.ncsu.edu/resources/comparative-study-of-chemical-pretreatments-of-dairy-manure-for-enhanced-biomethane-production/>. *BioRes.* 12(4), 7363-7375. oen
59. Yin, F., Dong, H., Zhang, W., Zhu, Z., & Shang, B. (2020). Additional function of pasteurisation pretreatment in combination with anaerobic digestion on antibiotic removal. *Bioresource Technology*, 297, 122414. <https://doi.org/10.1016/j.biortech.2019.122414>