

## Research Article

# Physicochemical Characterization and Biogas Potential assessment of Three Organic Substrates

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## Abstract

This study evaluates the biogas and methane production potential of three organic substrates, namely slaughterhouse waste, camel dung, and brewery waste (brewers' spent grain), to assess their suitability for anaerobic digestion and renewable energy production. The evaluation was based on the physicochemical characterization of the substrates, including organic matter content and carbon-to-nitrogen (C/N) ratio, which are recognized as key factors influencing microbial activity, process stability, and methane generation. Fresh samples were analyzed using standardized laboratory methods to estimate theoretical biogas potential together with expected biogas and methane yields. Results show that physicochemical composition strongly affects methanogenic performance. Camel dung exhibited high organic matter content and a favorable C/N ratio (20.2), resulting in a methane yield of 111 Nm<sup>3</sup>CH<sub>4</sub>/t FM and good process stability. Its balanced nutrient composition provides favorable conditions for methanogenic microorganisms, promoting efficient organic matter degradation and stable methane production. These characteristics identify camel dung as the most suitable substrate for anaerobic digestion among those investigated. In contrast, brewery waste showed the highest theoretical biogas potential (650 LN/kg OM) due to its high biodegradable organic matter content. However, its low C/N ratio (5.4) increased the risk of ammonia inhibition, limiting methane yield to 75 Nm<sup>3</sup>CH<sub>4</sub>/t FM despite its considerable energy potential. This finding demonstrates that a high theoretical biogas potential does not necessarily result in high methane production when nutrient balance is unfavorable for microbial metabolism. Although slaughterhouse waste had an optimal C/N ratio (23.3), it produced the lowest methane yield (32 Nm<sup>3</sup>CH<sub>4</sub>/t FM), suggesting lower biodegradability. This reduced performance may be associated with the presence of protein- and lipid-rich compounds capable of generating inhibitory intermediates that negatively affect methanogenic activity and methane conversion efficiency. Overall, camel dung appeared to be the most balanced substrate, while spent grain represents a promising energy-rich co-substrate and slaughterhouse waste a complementary feedstock for co-digestion. Their complementary characteristics suggest that co-digestion could optimize the C/N ratio, improve process stability, and enhance methane production. Analyses were conducted at the Biogas PlanET France laboratory.

## Keywords

Anaerobic Digestion, Methane Production, Organic Substrates, Carbon-to-nitrogen (C/N) Ratio, Co-digestion

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**Received:** 20 July 2026; **Accepted:** 5 August 2026; **Published:** 24 August 2026



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## 1. Introduction

Anaerobic digestion is a widely recognized biological technology for the treatment and energy recovery of organic waste [1]. It converts biodegradable organic matter into biogas, mainly composed of methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ), through the successive action of different groups of microorganisms [2]. This technology combines waste management with renewable energy production, contributing to greenhouse gas emission reduction by replacing fossil fuels in electricity and heat generation. After upgrading to increase its methane content, biogas can also substitute natural gas in gas distribution networks and the transport sector [3, 4].

The performance of anaerobic digestion strongly depends on the physicochemical and biochemical characteristics of the treated substrates. Parameters such as dry matter content, organic matter fraction, carbon biodegradability, biochemical composition, and the carbon-to-nitrogen (C/N) ratio directly affect process stability and methane yield [5]. Imbalances in these parameters may lead to inhibitory effects, particularly through ammonia accumulation or volatile fatty acid build-up, thereby reducing methanogenic activity [6].

Animal manure exhibits different physicochemical properties depending on animal species, feeding practices, and manure management systems, all of which influence methane production potential [7]. Livestock manure generally falls within the recommended C/N ratio range for anaerobic digestion (15-30) [8]. In addition, moisture content and total solids concentration are key factors affecting methane production, as these characteristics vary considerably according to manure collection and handling methods [9].

Organic wastes from diverse sources have attracted increasing interest as feedstocks for anaerobic digestion. Slaughterhouse waste presents a high energy potential because of its richness in organic matter, although its biodegradability may be limited by the complexity of its organic compounds. Camel dung is commonly used in anaerobic digestion due to its favorable nutrient balance and its ability to maintain process stability [10]. Brewery waste (spent grain) is also considered a promising substrate because of its high organic content and significant energy potential.

The biochemical composition of substrates, particularly the proportions of carbohydrates, proteins, and lipids, directly influences methane yield and substrate biodegradability. Lipid-rich substrates generally exhibit higher methane production potential because of their high energy density [11, 12]. When experimental digestion tests are unavailable, the theoretical methane potential estimated from physicochemical and biochemical characteristics provides a reliable approach for assessing substrate suitability in anaerobic digestion projects [13].

Therefore, this study aims to evaluate and compare the biogas and methane production potential of three organic substrates: slaughterhouse waste, camel dung, and brewery waste

(spent grain). Experimental analyses carried out at the Biogaz PlanET France laboratory were used to investigate the influence of substrate physicochemical characteristics on methanogenic performance. The objective was to identify the most suitable substrate and assess the potential of these feedstocks for maximizing methane production within anaerobic digestion and co-digestion strategies.

## 2. Materials and Method

### 2.1. Sample Description

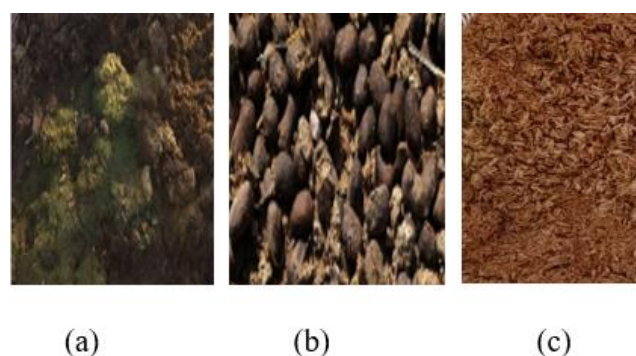
Three types of organic substrates were investigated in this study. The substrates were collected from three distinct sites located in the city of N'Djamena, selected based on their availability and potential for biogas production.

Slaughterhouse waste (Figure 1a) was collected from the Walia slaughterhouse, located in the Walia area in southern N'Djamena (12.081814 °N, 15.095276 °E). Sampling was carried out directly at the site to ensure representativeness and minimize any alteration of the material.

Camel dung (Figure 1b) was collected from a *férík* (nomadic livestock camp) also situated in southern N'Djamena (12°03'45.4" N, 15°06'16.5" E). This site was selected due to the abundance of this resource within local pastoral systems and its recognized methanogenic potential.

Finally, brewery waste (brewers' spent grain) (Figure 1c) was collected from the Farcha brewery, located in the industrial zone of the Farcha district in N'Djamena (12°07'51.8" N, 15°00'49.2" E). These lignocellulosic residues generated during the brewing process are of particular interest because of their high content of biodegradable organic matter.

All samples were collected following a rigorous sampling protocol designed to preserve their initial physicochemical characteristics prior to laboratory analyses.



**Figure 1.** (a) Slaughterhouse waste, (b) Camel dung, and (c) Brewery waste (brewers' spent grain).

## 2.2. Methods

### 2.2.1. Sample Preparation

The substrates were collected in their fresh state and subsequently homogenized to ensure analytical representativeness. Slaughterhouse waste was mechanically ground to reduce particle size and improve the accessibility of organic matter to microorganisms. Camel dung was cleaned of coarse impurities (sand and stones) prior to homogenization. Brewery waste (brewers' spent grain) was stored under controlled conditions to minimize biological degradation before analysis. Sample preparation and homogenization constitute essential steps to ensure the reliability of physicochemical analyses and the estimation of methanogenic potential.

### 2.2.2. Physicochemical Analyses

The analyses were performed on fresh matter (FM), dry matter (DM), and organic matter (OM) using standardized methods. The parameters analyzed included: dry matter (DM), organic matter (OM), total nitrogen, ammoniacal nitrogen, total organic carbon, total lipids, and total carbohydrates. These parameters are essential for characterizing substrates intended for anaerobic digestion and for estimating their theoretical methane production potential [12].

### 2.2.3. Carbon-to-Nitrogen Ratio (C/N)

The carbon-to-nitrogen (C/N) ratio is a fundamental indicator of a substrate's suitability for anaerobic digestion. A balanced C/N ratio promotes efficient microbial activity. Carbon serves as the primary energy source for anaerobic microorganisms, whereas nitrogen plays a crucial role in microbial growth and biomass synthesis. The C/N ratio reflects the overall nitrogen availability, the accumulation of ammoniacal nitrogen released during digestion, the formation of volatile fatty acids within the digester, and the nutrient status of the feedstock. Microorganisms require an appropriate C/N ratio for their metabolic processes; therefore, anaerobic digestion performance is highly sensitive to this parameter. According to most studies reported in the literature, the optimal C/N ratio ranges from 20:1 to 30:1, with a value of approximately 25:1 generally considered ideal for bacterial growth and process stability [14].

### 2.2.4. Estimation of Theoretical Biogas Potential

The theoretical biogas potential corresponds to the maximum volume of biogas that can be produced from biodegradable organic matter. This potential can be estimated from the biochemical composition of the substrate using conversion coefficients established in the scientific literature. [15] Several studies have demonstrated that the biochemical composition of substrates strongly influences methane production.

### 2.2.5. Biogas Yield

Biogas yield was expressed as  $\text{Nm}^3 \cdot \text{t}^{-1}$  of fresh matter and calculated from the specific biogas production potential relative to the organic matter content of the substrate. This approach is based on the principle that biogas production is proportional to the biodegradable organic fraction, generally expressed as volatile solids, and that the methanogenic potential can be estimated from the biochemical or elemental composition of the substrate using stoichiometric relationships or conversion coefficients reported in the literature [16].

### 2.2.6. Methane Yield

Methane yield was determined from the total volume of biogas produced while taking into account the volumetric fraction of methane, according to the following relationship:

$$R_{CH_4} = R_{Biogaz} \times F_{CH_4} \quad (1)$$

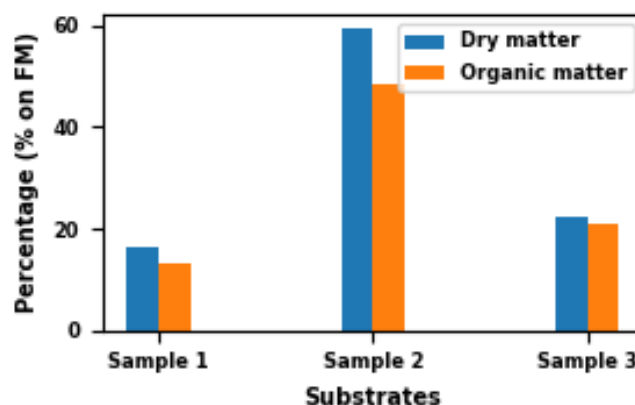
Where:

- 1)  $R_{CH_4}$  is the methane yield,
- 2)  $R_{Biogaz}$  is the biogas yield, and
- 3)  $F_{CH_4}$  is the volumetric methane fraction.

This method is widely used to express the actual energy production potential of biogas. Methane content generally ranges from 45% to 75%, with typical values between 50% and 70%, depending on the substrate characteristics and the operating conditions of the anaerobic digestion process [17].

## 3. Results and Discussion

### 3.1. Physicochemical Characterization and Biochemical Properties of the Investigated Substrates



**Figure 2.** Distribution of dry matter and organic matter content in the substrates: slaughterhouse waste (Sample 1), camel dung (Sample 2), and brewer's spent grain (Sample 3).

Figure 2 presents the physicochemical characterization of

the substrates, which is essential for assessing their suitability and potential for anaerobic digestion. Camel dung (Sample 2) exhibited the highest dry matter content (approximately 59%) and organic matter content (approximately 48%). These high values are associated with its richness in lignocellulosic fibers, which may, however, slow down the biodegradation process in the absence of appropriate pretreatment. [18]. Nevertheless, co-digestion has been identified as an optimal strategy for enhancing biogas production, improving process stability through pH regulation, and reducing the risk of inhibitory effects during anaerobic digestion [19].

The results indicate that camel dung exhibits the highest organic carbon content (approximately 27%), followed by brewery waste (approximately 11%) and slaughterhouse waste (approximately 7%). The high carbon content observed in animal manure may be attributed to the presence of lignocellulosic residues derived from partially digested plant fibers, particularly cellulose and hemicellulose [20]. Consequently, animal manures are considered substrates rich in biodegradable organic matter and are widely used in anaerobic digesters either as primary feedstocks or as biological inocula.

However, the presence of lignocellulosic compounds may slow down the hydrolysis stage, which is often regarded as the rate-limiting step in anaerobic digestion. Recent studies have shown that fiber-rich substrates frequently require optimization strategies, such as co-digestion or pretreatment processes,

to enhance their biodegradability and methane production performance.

Brewery waste (brewers' spent grain) exhibits an intermediate organic carbon content. This agro-industrial residue is mainly composed of fibers, proteins, and fermentable organic compounds, making it a promising substrate for biogas production. Several recent studies have reported that the anaerobic digestion of brewers' spent grain can achieve high methane yields and improve organic matter removal efficiency when co-digested with other organic substrates [21].

Slaughterhouse waste shows the lowest organic carbon content in Figure 2. This may be explained by its high moisture content and its composition, which is predominantly characterized by proteins and lipids rather than structural carbon compounds. Nevertheless, this waste stream possesses a high energy potential, since lipid degradation can generate substantial amounts of methane.

Table 1 presents the results of the physicochemical analysis of the different substrates investigated in this study. It summarizes the parameters measured for each sample analyzed in the laboratory. To facilitate data interpretation and comparison, each set of results is associated with a specific substrate type. Sample 1 corresponds to slaughterhouse waste, Sample 2 represents camel dung, and Sample 3 refers to brewery waste (brewers' spent grain). All results are summarized in the table below.

**Table 1.** Organic carbon content, total nitrogen content, and C/N ratio of the studied substrates.

| Substrates | Organic Carbon (C) | Total Nitrogen (N) | C /N Ratio |
|------------|--------------------|--------------------|------------|
| Sample 1   | 7                  | 0,3                | 23,3       |
| Sample 2   | 27,3               | 1,35               | 20,2       |
| Sample 3   | 11,4               | 2,11               | 5,40       |

The characterization of organic carbon (C), total nitrogen (N), and the C/N ratio constitutes a key indicator of anaerobic digestion performance. The results show that camel dung (Sample 2) exhibits the highest carbon content (27.3%) and a balanced C/N ratio (approximately 20.2), which is favorable for methanogenic microbial activity [21]. Indeed, a C/N ratio ranging from 20 to 30 is generally considered optimal for ensuring stable biogas production and preventing nutrient limitations.

Slaughterhouse waste (Sample 1) exhibits a C/N ratio of 23.3, which also falls within the optimal range, despite its lower absolute carbon and nitrogen contents. This suggests a suitable nutritional balance for microbial growth. However, its high protein and lipid contents may lead to the formation of ammonia and inhibitory fatty acids when the organic loading rate is excessive [22]. Recent studies have shown that these

substrates often require co-digestion to mitigate such inhibitory effects and improve process stability.

In contrast, brewery waste (Sample 3) exhibits a very low C/N ratio (approximately 5.40), indicating a relative excess of nitrogen. This condition is unfavorable for anaerobic digestion because it promotes the accumulation of free ammonia, which inhibits methanogenic archaea and reduces methane yield [23]. Recent studies further confirm that C/N imbalances can result in a significant decrease in biogas production and compromise process stability [23, 24].

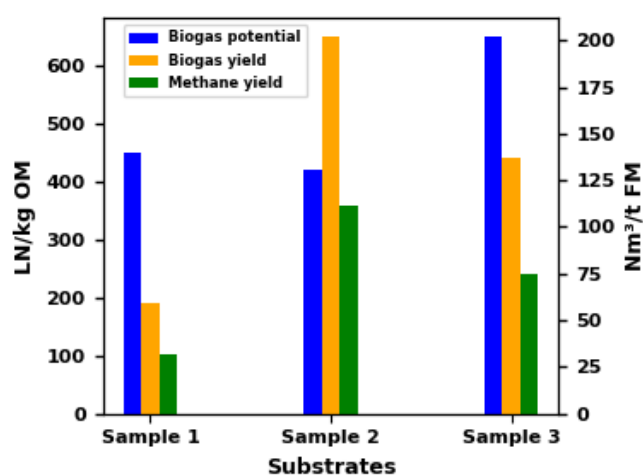
The comparative analysis highlights a clear complementarity among the substrates. Camel dung, which is rich in carbon and fibrous materials, can compensate for the carbon deficiency of brewery waste, while slaughterhouse waste provides readily biodegradable energy-rich compounds. This synergy forms the basis of co-digestion, which is widely recognized as

an effective strategy for optimizing biogas yield. Recent research has demonstrated that adjusting substrate mixtures can significantly enhance methane production and improve the overall performance of anaerobic digesters [23].

The results presented in Table 1 indicate that only Samples 1 and 2 provide conditions favorable for stable anaerobic digestion, whereas Sample 3 requires adjustment through co-digestion. Therefore, combining these substrates represents a relevant approach for optimizing the C/N ratio and maximizing biogas production.

### 3.2. Comparative Analysis of Biogas Potential and Methanization Performance of the Investigated Substrates

The results presented in Figure 3 reveal significant differences among the three substrates in terms of biogas potential, biogas yield, and methane yield. These variations primarily reflect differences in the biochemical composition and biodegradability of the substrates, which are recognized as key factors governing anaerobic digestion performance [25].



**Figure 3.** Comparison of the biogas potential (blue), biogas yield (orange), and methane yield (green) of the three substrates.

Sample 1 exhibited a moderate biogas potential (~450 LN/kg OM), associated with a relatively low biogas yield (59 Nm³ CH<sub>4</sub>/t FM) and a limited methane yield (32 Nm³ CH<sub>4</sub>/t FM) compared with the other substrates. This overall poor performance suggests a reduced biodegradability of the organic matter, likely due to the presence of complex or poorly hydrolysable compounds. Indeed, the accumulation of compounds such as long-chain fatty acids may inhibit methanogenic activity and reduce conversion efficiency [26].

In contrast, Sample 2 showed the highest biogas and methane yields, reaching approximately 202 Nm³/t FM and 111 Nm³ CH<sub>4</sub>/t FM, respectively, despite having a biogas potential of about 420 LN/kg OM. This observation reflects excellent

biodegradability and a high efficiency of organic matter conversion. These performances may be attributed to a composition rich in readily biodegradable substrates and to environmental conditions favorable for microbial activity. Recent studies have demonstrated that the adaptation of microbial communities can improve methane yields by nearly 25%, highlighting the importance of biological factors in the anaerobic digestion process [26].

Sample 3 exhibited the highest biogas potential (~650 LN/kg OM), but intermediate biogas and methane yields (137 Nm³ CH<sub>4</sub>/t FM and 75 Nm³ CH<sub>4</sub>/t FM) were respectively, compared with Sample 2. This discrepancy between theoretical potential and actual yield indicates the presence of refractory organic fractions, particularly lignocellulosic compounds, which limit hydrolysis and methane conversion. This behavior is consistent with recent literature reporting that lignocellulose-rich substrates often require pretreatment to improve digestibility and enhance methane production [27].

## 4. Conclusions

This study aimed to evaluate the methanogenic potential of three distinct organic substrates, namely slaughterhouse waste, camel dung, and brewery waste (brewers' spent grain). The assessment was based on their physicochemical characterization and the theoretical estimation of their biogas production potential. The results highlight the decisive role of biochemical composition and the carbon-to-nitrogen (C/N) ratio in determining both the stability of the anaerobic digestion process and the efficiency of methane production.

The comparative analysis revealed that camel dung provides the most favorable conditions for anaerobic digestion. Its balanced C/N ratio (approximately 20.2) falls within the optimal range for methanogenic microbial activity. This substrate also exhibited the highest biogas and methane yields, reaching 202 Nm³/t FM and 111 Nm³ CH<sub>4</sub>/t FM, respectively, indicating stable and efficient biological conversion.

In contrast, brewery waste displayed the highest theoretical biogas potential owing to its high content of biodegradable organic matter. However, its low C/N ratio (approximately 5.4) indicates an excess of nitrogen, which may lead to ammonia accumulation, causing partial inhibition of methanogenic microorganisms and consequently limiting the effective methane yield.

Although slaughterhouse waste exhibited a C/N ratio within the optimal range, it showed the lowest methanogenic performance. This can be explained by the presence of proteinaceous and lipid-rich compounds whose degradation may generate inhibitory substances such as ammonia and long-chain fatty acids.

Overall, the results demonstrate a functional complementarity among the investigated substrates. Camel dung contributes to the biological stability of the process, brewery waste provides a high energy potential, and slaughterhouse waste serves as an additional source of organic matter. Therefore,

co-digestion appears to be a promising strategy for optimizing the C/N ratio, improving biodegradability, and maximizing biogas production.

## Abbreviations

FM Fresh Matter  
DM Dry Matter  
OM Organic Matter

## Supplementary Material

The supplementary material can be accessed at <https://doi.org/10.11648/j.ajep.20261504.14>

## Acknowledgments

At the completion of this work, we would like to express our sincere gratitude to Arthur Benoist, Sandra Vieuxloup, and Elisabeth Duval of the PlanET Biogas Laboratory, France, for their availability, attentive support, and prompt responses to our requests. We are especially grateful for their valuable contribution in conducting the laboratory analyses and for their insightful assistance in the interpretation of the results and the scientific analysis of this study.

## Author Contributions

**Abdoulaye Affadine Al-hafiz:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing

**Younous Ali Ahmat:** Data curation, Project administration, Visualization

**Mahamat Abdallah Ban-nah:** Formal Analysis

**Boukar Abdelhakim:** Validation, Supervision

**Serigne Abdoul Aziz Niang:** Software

**Adoum Abdraman Mahamat:** Investigation

## Data Availability Statement

The data is available from the corresponding author upon reasonable request.

## Conflicts of Interest

The authors declare no conflicts of interest.

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## Biography



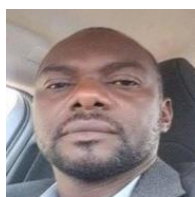
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## Research Field

**Abdoulaye Affadine Al-Hafiz:** Biogas, Biomass, Chemistry, Energy, Climate, Bioenergy

**Younous Ali Ahmat:** Energy, Climate, Biomass, Bioenergy, Aerosol

**Mahamat Abdallah Ban-Nah:** Mechanic, Energy, Bioenergy, Climate, Chemistry

**Boukar Abdelhakim:** Mechanic, Energy, Biomass, Bioenergy, Chemical Process Engineering

**Serigne Abdoul Aziz Niang:** Energy, Climate, Biomass, Bioenergy, Aerosol

**Adoum Abdraman Mahamat:** fluid mechanics, Energy, Climate, Bioenergy, Chemical Process Engineering