

# Determination of Biochemical Methane Potential Under Mesophilic Conditions and Realistic Energy Estimation on Industrial Scale

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## Abstract

The aim of this study was to determine the Biochemical Methane Potential (BMP) of some of the raw materials mainly used in the Biogas Plant: chicken manure, cow manure, chopped maize straw, vinasse 17 brix, beet tail, and wheat grain (wheat starch) under laboratory conditions.

During the experimental study, the dry matter and volatile solid contents of the raw materials were calculated and the amounts added to the reactors as inoculum solution were determined. The reactors were operated under mesophilic conditions by monitoring their temperatures during the 30-day operating period. The BMP where the experimental study was carried out consists of six reactors, CO<sub>2</sub> and H<sub>2</sub>S absorption units, and a methane gas measurement unit for the released methane gas. During the experimental study, wheat starch was used as the reference material and the inoculum solution fed to the reactors was used as the test solution. In the study where methane gas outputs were determined, the value reached for wheat starch was 363.15 Nml CH<sub>4</sub>/gVS, for chicken manure it was 260.83 Nml CH<sub>4</sub>/gVS, for cow manure it was 156.08 Nml CH<sub>4</sub>/gVS and for chopped straw it was 177.42 Nml CH<sub>4</sub>/gVS. In addition, in order to determine the methane gas output value in the BMP device, vines were studied as a sugar factory waste, and the methane gas output value was determined as 372.57 Nml CH<sub>4</sub>/gVS.

The study was carried out by taking into account the daily degradation rates of each raw material and calculating the degradation rates as percentages from the first day to the last day.

In the 30-day BMP analysis based on the daily degradation of each feedstock in anaerobic digestion, it was seen that the gross energy obtained from real biogas reactors was realized at 94%, showing a very close estimate. In addition to theoretical values, it is thought that analyzing a wide range of wastes in the laboratory environment will shed more light on biogas installation feasibility studies. Since the hydraulic retention time (the number of days the material remains in the digester) of real biogas digesters is 28 days, this period is taken as the basis.

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**Categories:** Fossil Fuel Energy, Renewable Energy in Agriculture

**Keywords:** anaerobic digestion, biochemical methane potential, mesophilic conditions, positive control, negative control

## Introduction

Agricultural waste, manure, municipal trash, plant material, sewage, green waste, wastewater, and food waste are among the raw materials used to create biogas, a gaseous renewable energy source [1]. Anaerobic digestion using anaerobic organisms or methanogens within a bioreactor, bioreactor, or anaerobic digester produces biogas [2]. Methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) make up the majority of the gas's makeup, with trace amounts of hydrogen sulfide (H<sub>2</sub>S), siloxanes, and moisture (Table 1). The biogas formed in the anaerobic environment is converted into electrical energy by cleaning the H<sub>2</sub>S in it through a series of treatment processes and burning it in gas combustion engines. An anaerobic digester that breaks down agricultural waste or energy crops is commonly referred to as a biogas plant.

In most of the known biogas producers, the temperature demand is in the temperature range between 37 and 42°C. Operating in mesophilic conditions, plants are the most common in practice, because in this temperature range, a high gas efficiency and good process stability are achieved [3].

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Compound	Formula	%
Methane	CH <sub>4</sub>	50-75
Carbon dioxide	CO <sub>2</sub>	25-50
Nitrogen	N <sub>2</sub>	0-10
Hydrogen	H <sub>2</sub>	0-1
Hydrogen sulfide	H <sub>2</sub> S	0-3
Oxygen	O <sub>2</sub>	0-0
Siloxanes	n R <sub>2</sub> Si(OH) <sub>2</sub>	Trace
Water	H <sub>2</sub> O	Saturation

**TABLE 1: Biogas composition**

All organic substances have the potential to be used for biogas production. Numerous studies have been conducted on biogas production from various organic wastes. The method used for biogas production is the decomposition process in an oxygen-free environment. Chulalaksananukul and his colleagues investigated the production of biogas from pineapple peels in their study and determined the maximum gas production conditions. They studied several parameters affecting biogas production. They conducted their studies in laboratory-type 6 L reactors and at approximately 30°C. Chulalaksananukul and his colleagues stored the pineapple peels they used in their studies at +4°C. Then, they brought the dimensions of the peel to an average of 1 cm before feeding the reactor. The working volume of the reactor was determined as 4.8 L and the raw material was placed in the reactor up to this value. The reactors were kept at ambient temperature for 30 days by mixing twice a day. The pH and C/N ratio were kept under control throughout the experiment. In their analyses, the pH value of the pineapple peel was found to be 4.6. Chulalaksananukul and his colleagues worked with both this pH value and the raw material at pH 7, and biogas production was better in the group with pH 7 [4].

Since the nitrogen content of pineapple peel is quite low (0.7%), the C/N ratio is high. This value is not suitable for biogas production. Nitrogen can be added to the system inorganically or organically. Animal manure or urea can be used as an organic nitrogen source. In this study, urea was used as a nitrogen source. In this study, a group of fed-type reactors were also used and the results were compared. The best feeding rate in the fed-type reactor was determined as 1 kg/m<sup>3</sup> day.

In this study, Chulalaksananukul and his colleagues concluded that:

- Batch-type reactors are more effective than fed-type reactors in biogas production.
- Optimization studies should be conducted for fed-type reactors.
- Using pineapple peels in biogas production has advantages both in terms of the environment and being green energy [4].

Velmurugan and Ramanujam investigated the amount of biogas and methane that can be obtained by decomposing vegetable waste in an anaerobic environment. The experiments were carried out in a laboratory-type, fed-type biogas reactor at 35°C, a 2.25 g/L.day organic loading rate, and 30 days hydraulic holding time. The results of this study by Velmurugan and Ramanujam are as follows: the average methane content of the produced biogas is 65%, and the methane potential of vegetable waste is 0.387 CH<sub>4</sub>/g VS [5]. In the studies conducted, a single material can be used for anaerobic decomposition process, or biogas can be produced by mixing more than one material. This method can be useful in facilities with material shortages. The mixture ratio at which maximum biogas is obtained is determined by mixing more than one material in different ratios, and biogas production is continued over this ratio.

Lin et al. investigated the amount of methane that can be produced from fruit and vegetable waste and food waste in their study. They conducted their experiments in a laboratory type continuous feed reactor at 35°C and selected the organic loading rate as 3 kg VS/m<sup>3</sup> day. While fruit and vegetable waste has methane of 0.3 m<sup>3</sup>CH<sub>4</sub>/kg VS and 59.3% decomposition rate, food waste has methane of 0.56 m<sup>3</sup>CH<sub>4</sub>/kg VS

and 85.6% decomposition rate. Fruit and vegetable waste produces 2.17 m<sup>3</sup> day biogas and 0.42 m<sup>3</sup>CH<sub>4</sub>/kg VS methane. However, methane production from food waste is prevented due to acid accumulation. In this study, the amount of methane that can be obtained by mixing fruit and vegetable waste and food waste in different proportions and its stabilization were also investigated. Lin and his colleagues produced biogas by mixing fruit and vegetable waste and food waste in three different ratios: 2:1, 1:1, and 1:2 [6].

Amon and his colleagues conducted a study on biogas production using daily cattle manure and corn. They conducted the study in 1 L reactors for 60 days at 38°C. Cattle manure and corn were used separately for biogas production. Amon and his colleagues divided the cattle into three categories (low, medium, and high milk yield) according to their milk yield and subjected each category to two repeated biogas tests. They also examined the structure of each group of manure.

Here, dairy farms-1 and 2 are low milk yield, 3 and 4 are medium milk yield, 5 and 6 are high milk yield cattle. In the same study, different conditions affecting the amount of biogas obtained from corn were also evaluated. These conditions include silage effect, harvest time, harvest technology, and so on. Amon and his colleagues obtained the maximum methane output from balanced-fed cattle with medium milk yield and concluded that the anaerobic decay of animal manure is affected by its nutrition and performance. They concluded that turning corn into silage increases the amount of biogas obtained [7].

Li et al. conducted a study on the amount of biogas obtained from different mixing ratios of corn and chicken manure in wet, semi-solid and solid forms. In the study, corn and chicken manure were mixed in the ratios of 1:0, 3:1, 1:3, 0:1 and the biogas outputs were compared. The results obtained show that the combined anaerobic digestion of corn and chicken manure increased the biogas output. Li et al. obtained the best result as 218.8 mL/g VS in 3:1 corn-chicken manure mixture and wet anaerobic digestion [8]. Kafle et al. also investigated the effect of the mixing ratio on biogas production by mixing pig manure and Brassica rapa (Chinese cabbage) waste silage in different ratios. In this study, they produced biogas by mixing pig manure and Chinese cabbage waste silage in the ratios of 100:0, 75:25, 67:33, 33:67, 0:100, respectively [9].

Animal manure can be used alone in biogas production, as well as as an inoculum for different organic wastes. In another research study, they used cattle manure and activated sludge as inoculum for corn stalk and wheat straw waste and compared the amount of biogas produced. They used only the biogas content of cattle manure as a control group [10].

In the absence of free oxygen, organic matter, food, plant debris, animal manure, sewage sludge, and biodegradable portions of municipal solid waste typically break down to produce a gas that contains 40-70% methane, with the remaining gas being primarily carbon dioxide and traces of other gases. Like compressed natural gas (CNG) or liquefied petroleum gas (LPG), this gas burns cleanly when ignited, meaning it does not smoke or emit any disagreeable odors. It is frequently referred to as "biogas," which is an ambiguous and incomplete phrase because, like other biogas, the gas produced by aerobic decomposition (carbon dioxide) is also referred to as "biogas" because it is a byproduct of biodegradation. The combustible CH<sub>4</sub>-CO<sub>2</sub> mixture (as well as traces of other gases) produced by the anaerobic breakdown of organic materials is referred to as "biogas" in some contexts. Compared to LPG and CNG, biogas has a lower calorific value, although it is still good. Under anaerobic conditions, biogas is spontaneously created from biogenic substances-in fact, from any biomass. When released into the atmosphere, methane, the primary component of this naturally occurring biogas, significantly contributes to global warming. Methane has emerged as one of the most used fuels for transportation, heating, and energy production during the last century. Although natural gas now provides the great majority of the methane utilized in society, interest in extracting methane from decomposing biomass is growing quickly. This does not imply that all methane emitted by natural sources must be captured. It implies that a fully contained, regulated, and optimized biogas process, along with the utilization of nature's capacity to create environmentally sound, renewable gaseous biofuels, can be developed into an economically successful enterprise [11].

Anaerobic fermentation of organic matter produces biogas, which normally contains 55-70% CH<sub>4</sub> (though it can be greater), 30-45% CO<sub>2</sub>, and a few trace amounts of water and hydrogen sulfide. Anaerobic digestion is appropriate for animal manure, sewage sludge, industrial wastes, slaughterhouse wastes, agricultural wastes like corn and grass, and the organic portion of municipal solid waste. The type of waste feed determines the composition of biogas. For instance, biogas derived from chicken manure includes 60-80% methane (CH<sub>4</sub>), whereas biogas derived from grass waste has about 55%. Slurry, which is the digested organic debris that remains after the anaerobic fermentation process, can be utilized in the place of traditional manufactured fertilizer [12].

The most effective method of turning wastes with a dry matter concentration of less than 30% (preferably between 5.5% and 12.5%), such as food scraps, sewage sludge, manure, and other organic wet wastes, into energy is anaerobic digestion. With anaerobic fermentation technology, moist organic wastes are fed into a reactor and fermented anaerobically to produce carbon dioxide and methane, which can then be utilized

as fuel for vehicles, for heating, or to generate power. Dry anaerobic fermentation techniques are now being marketed for dry matter concentrations of at least 30%. Biomass plants are used in Germany's feedstock mix. In anaerobic digestion, microorganisms ferment organic materials without oxygen in a heated, sealed vessel called a fermentor. Heat recovered from a methane-fired boiler or gas engine system powers the fermentor. The temperature of the fermenter tank can be raised to either the thermophilic range (55–65°C), where the retention period is usually 12–15 days, or the mesophilic range (30–35°C), where the feed waste usually stays in the fermenter for 15–30 days. Although thermophilic fermentation systems produce more methane, they also come with higher running and monitoring costs, more energy input, and more costly technology. Longer residence times result in higher conversion rates, with biogas (methane and carbon dioxide) typically accounting for 30–60% of the mass of input biomass solids. Enzymatic hydrolysis is the initial stage of decomposition, during which lipids are converted to fatty acids, proteins into amino acids, and carbohydrates into sugars. The primary usable energy output of anaerobic digestion, methane, is produced by methanogenic bacteria using hydrogen, CO<sub>2</sub>, and volatile fatty acids [13]. In anaerobic fermentation, organic waste is fermented by bacteria without the presence of free oxygen. Complex biodegradable organics are broken down in four steps by fermentation.

1. Hydrolysis breaks down large protein macromolecules, lipids, and carbohydrate polymers (such as cellulose and starch) into sugars, long-chain fatty acids, and amino acids.

2. During acidogenesis, these products undergo fermentation, mostly yielding volatile fatty acids including propionic, butyric, valeric, and lactic acids.

3. These fermentation products are consumed by bacteria during acetogenesis, which results in the production of hydrogen, carbon dioxide, and acetic acid.

4. To create methane, methanogenic organisms use hydrogen, acetate, and a small amount of carbon dioxide. To do this, methanogenic bacteria employ three metabolic pathways:

a. Acetotropic pathway ( $4\text{CH}_3\text{COOH} \rightarrow 4\text{CO}_2 + 4\text{CH}_4$ ),

b. Hydrogenotropic pathway ( $\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$ ),

c. Methylotropic pathway ( $4\text{CH}_3\text{OH} + 6\text{H}_2 \rightarrow 3\text{CH}_4 + 2\text{H}_2\text{O}$ ) [14].

Anaerobic fermentation has the following advantages:

1. Natural, plentiful waste sources are used.

2. It uses a lot less land than landfill disposal or aerobic composting.

3. It lowers the weight and volume of waste that must be dumped in landfills.

4. The process produces net energy.

5. The high-quality renewable fuel it generates has found practical use in numerous applications.

6. It takes the place of fossil fuels and eliminates infections.

7. It significantly lowers emissions of the powerful greenhouse gases methane and carbon dioxide.

8. It significantly reduces smells.

9. It is capable of producing both a nutrient-rich liquid fertilizer and clean compost.

10. It makes the majority of materials as recyclable as possible.

11. Compared to other treatment methods, it is far more cost-effective over the course of its life cycle [14].

The anaerobic fermentation method has certain drawbacks, much like any other biotechnological step. The most significant is that it does not break down lignin, which is a crucial part of wood. This is probably due to the fact that anaerobic bacteria are released even before oxygen, which prevents young woody plants from having a chance to adapt. Nevertheless, producers and researchers have found success using woody biomass, marine plants, and aquaculture crops as anaerobic fermentation fuel. Because anaerobic bacteria are either poorly developed or not grown at all in low-temperature conditions, the anaerobic fermentation process likewise performs worse there. In contrast, the procedure is less troublesome in warmer, tropical regions. Producers in cooler climates must therefore heat the reactors. Biogas production

slows considerably when the temperature within a reactor falls below 20°C, and fermentation stops when the reactor approaches freezing point [15].

Any biomass containing protein, cellulose, hemicellulose, lipids, or carbohydrates can generally be used to produce biogas [16]. It is very important to choose a fermentation technique that works well for the given biomass. The ratios of total solids (TS) and volatile solids (VS) are the most important factors that directly affect biogas production [17]. Methane bacteria in the digestive systems of animals cannot metabolize the compounds found in their feces [18]. Some of the methane in cattle feces is converted during animal digestion, which reduces the efficiency of the biogas produced [4]. Methane is also found in cattle waste after digestion [19]. This shows that it is suitable for biogas inoculation. However, if inoculation is not done, fermentation may not start for months even if leaves, agricultural waste, industrial waste, and straw are fermented [20]. The high fiber content of cattle manure and the difficult fermentation process are its disadvantages [21]. Sugar mill wastes such as vinasse, depending on their dry matter content, do not experience such problems under mesophilic conditions during the fermentation process [22].

Through a process known as anaerobic digestion, a collection of bacteria collaborate to make biomethane. Biomethane, a renewable energy source, is produced by the anaerobic digestion process from household solid waste, industrial waste from agriculture, and/or sludge from wastewater treatment facilities [23]. The breakdown of organic materials without oxygen is the foundation of anaerobic digestion, a natural biological process [24]. One example of a closed system that produces methane from organic waste is a garbage landfill. Numerous studies have demonstrated that there are three primary phases to the anaerobic digestion process. Hydrolysis, acidogenesis, and methanogenesis are the names of these processes, respectively. Psychrophilic (12-16°C), mesophilic (35-37°C), and thermophilic (50-60°C) conditions can all lead to the synthesis of biomethane [25]. Hydrolysis is the initial stage in the production of biomethane. Bacteria transform complicated organic materials that are not dissolved into dissolved ones during this phase. In many anaerobic digestion procedures, the hydrolysis stage may be the phase that limits the pace of digestion. Chemical agents are utilized in certain industrial applications to speed up this step and the hydrolysis stage [26]. The end products of the hydrolysis step are transformed into simple organic acids, carbon dioxide, and hydrogen by the bacteria that produce acid in the second stage. At this point, acetic acid (CH<sub>3</sub> COOH), propionic acid (CH<sub>3</sub> CH<sub>2</sub> COOH), butyric acid (CH<sub>3</sub> CH<sub>2</sub> CH<sub>2</sub> COOH), and ethanol (C<sub>2</sub> H<sub>5</sub> OH) are the primary acids that are produced.

Using a variety of microbial activities, anaerobic digestion is a sophisticated natural biological process that may produce biogas from biomass (organic matter) that contains cellulose [27]. Numerous investigations to ascertain biomethane potentials have been documented in the literature [28,29]. Biomass waste is therefore regarded as one of the most significant renewable energy sources [28]. Alternative methods for recovering bioenergy, recovering plant nutrients, and lowering carbon emissions can be found in anaerobic digestion [29]. Lignocellulosic compounds found in animal manure limit the hydrolysis stage in anaerobic digestion. Addition of co-substrate helps to achieve stable anaerobic fermentation by improving nutrient balance, increasing buffering capacity, and diluting toxic compounds. However, it has been reported that biogas production amount and biomethane yield are increased in anaerobic fermentation by applying co-substrate strategies in cow dung and food waste [30].

This study mostly employed sewage sludge from breweries, abattoir waste, corn straw, cow dung, waste wheat, and sugar industry waste (vinasse).

The use of industrial wastes in biogas production can be considered an important industrial development within the framework of circular economy applications. The most important aspect of this study is that the studies on vinasse, which is intensively released in large industrial tonnage production facilities such as the sugar production industry and has been used in the energy sector until today in terms of dry matter content, are quite limited in the literature. This is because the use of vinasse, which is the subject of the study and has a dry matter content of 14%, in energy production is an important alternative presented to the producer, as it is determined that it is more effective and has a higher energy content than the use of starch and derivative raw materials, which are richer in terms of organic content.

## Materials And Methods

### Equipment

The target substrates' BMP was examined using the Automatic Methane Potential Test System II (AMPTS II, Bioprocess Control Sweden). A standardized laboratory setup created especially for the automatic BMP determination of any biodegradable material is the AMPTS II (Figure 1). Precalibrated flow cells that provide a signal for every 10 mL of generated gas are used to measure the gas through water displacement. At each measurement point, the gas volume is normalized to 0°C, 1 atm, and dry gas conditions using temperature and pressure sensors.



**FIGURE 1: The Automatic Methane Potential Test System II (AMPTS II, Bioprocess Control Sweden) BMP**

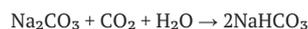
The factory at the Meram Renewable Energy Fermenter in Konya provided the inoculum.

## Materials

Sigma-Aldrich provided sodium hydroxide pellets, reagent grade 97%. We prepared a 3 M alkaline solution for CO<sub>2</sub> fixation by using wheat starch soluble (CAS No. 9005-25-8, Merck) and 0.4% thymolphthalein as a pH indicator.

## Efficiency of the CO<sub>2</sub>-Fixing Unit of AMPTS

A gas combination with a given concentration (i.e., 60% CH<sub>4</sub> and 40% CO<sub>2</sub>) was circulated through a 100 mL bottle holding an 80 mL 3 M NaOH solution at several flow rates (0.1-24 L/day) in order to examine the effectiveness of the CO<sub>2</sub>-fixing unit of the AMPTS system. While CO<sub>2</sub> was eliminated as a result of the chemical reactions, CH<sub>4</sub> made it to the gas flow measurement device:



Before and after the CO<sub>2</sub> fixing unit, samples were gathered, and Gas Chromatography was used for their analysis. It has been demonstrated that the NaOH unit can absorb 92% to 99% of CO<sub>2</sub>.

## Materials

The biochemical methane potential was investigated for eight individual samples: chicken manure, cattle manure, powdered maize straw, chopped maize straw, vinasse, waste wheat, beet tail, and brewery sludge. Starch is used as a standard compound. Starch is reported in literature as  $350 \pm 33$  [4,5].

## Experimental procedure

The biomass should be described in terms of TS and VS before to beginning any BMP test. All inorganic and organic constituents are referred to as dry matter (TS) and can be quantified using a standard procedure. In order to eliminate all water content from a biomass sample, the sample must be heated to 105°C. The chemical substances in the sample serve as VS's representative. To burn up the organic materials, the sample should be heated to 550°C for 2 hours after the TS measurement is complete. The VS content of the biomass is indicated by the weight difference between the sample after heating it to 105°C and 550°C.

The ratio of the initial amount of wet sample ( $m_{\text{Wet}}$ ) to the amount of dried sample ( $m_{\text{Dried}}$ ) is used to compute TS. In the BPC protocol, VS is calculated as the ratio of the amount of sample after drying and burning ( $m_{\text{Burned}}$ ) to the initial amount of sample. This yields an estimate of the amount of organic material in the sample and can be expressed as either the percent of the TS or the percent of the wet sample.

$$TS (\%) = \frac{m_{dried}}{m_{wet}} \quad (1)$$

where  $m_{dried}$  (gr) is the amount of the dried sample,  $m_{wet}$  (gr) is the initial amount of wet sample.

$$VS (\%) = \frac{m_{dried} - m_{burned}}{m_{wet}} \quad (2)$$

Where  $m_{burned}$  is the amount of (gr) of sample after burning.

The %DM, %ODM, and %VS values of the three primary waste sources for biogas-chicken manure, cow dung, and maize straw-for the initial study are listed in Table 2. The BMP gadget can typically study six parameters. However, only these five factors will be examined because the other material we are working with is not the focus of this paper. However, as the inoculum/substrate ratio of 2 is the subject of this study, studies were also carried out on the inoculum/substrate ratios of 3 and 4 (Tables 3 and 4).

Here, starch served as the positive control and the inoculum as the negative control.

	%DM	%ODM	%VS
Inoculum (negative control)	12,52	58,90	7,37
Chicken manure	33,99	62,09	21,10
Cow manure	17,53	81,83	14,34
Powdered maize straw	96	95,1	91,30
Starch (positive control)	98	99,9	97,9

**TABLE 2: First experiment for main raw materials used in biogas @ I/S = 3 (For determination of chicken manure, cow manure, powdered maize straw, and starch) (inoculum PH = 8.1)**

%DM, dry matter; %ODM, organic dry matter; %VS, volatile solids

	%DM	%ODM	%VS
Inoculum (negative control)	8,32	52,28	4,35
Vinasse 17 brix	17,1	73,1	12,5
Starch (positive control)	99,99	100	99,99

**TABLE 3: Second experiment for main raw materials used in biogas @ I/S = 4 (For determination of vinasse 17 brix as a sugar factory waste and starch)**

%DM, dry matter; %ODM, organic dry matter; %VS, volatile solids

	%DM	%ODM	%VS
Inoculum (negative control)	8,39	51,01	4,28
Beet tail	16,8	95,24	16,00
Starch (positive control)	89	90	80,10

**TABLE 4: Third experiment for main raw materials used in biogas @ I/S = 4 (for determination of beet tail and starch)**

%DM, dry matter; %ODM, organic dry matter; %VS, volatile solids

### Determining I/S (inoculum/substrate) ratio

Inoculum/substrate (I/S) ratio was taken as 3 in the first experiment. While this may vary according to the test method, the recommended I/S ratio is 2 and above. No general rule exists considering the choice of I/S exists, but a ratio  $\geq 2$  is commonly considered to provide stable and reliable conditions for anaerobic degradation.

The total amount of inoculum and substrate was determined to be 1,800 grams (approximately 1,800 ml). The total volume of the test bottles was 2,500 ml. By adding 1,800 grams, a 700 ml of gas accumulation space is left.

Here we find out how much of a 1,800 gram bottle will be inoculum and how much will be substrate.

Ratio 3:1, (I/S = 3),

$$\frac{m_{IS} \times V_{SI}}{m_{SS} \times V_{SS}} = 3 \quad (3)$$

$$m_{SS} + m_{IS} = 1800 \quad (4)$$

Where,

$m_{IS}$ , is the total amount of inoculum in the sample,

$m_{SS}$ , is the total amount of substrate in the sample,

$V_{SI}$ , is the volatile solid of the inoculum,

$V_{SS}$ , is the volatile solid of the substrate.

If we make an example calculation for chicken manure, in Table 2, volatile organic solids amounts were found for chicken manure. Substituting these values in Equation (3):

$$\frac{(1800 - m_{SS}) \times 7.37}{m_{SS} - 21.10} = 3$$

$m_{SS}$ , 187.72 grams chicken manure,  $(1,800 - 187.72) = 1,612.28$  grams inoculum added to the bottle. The weight (gram) values of the wastes used in the first experiment that should be added according to I/S = 3 were calculated as shown in Table 5.

	Amount of inoculum (g)	Amount of substrate (g)	%Volatile solid	I/S	Total amount of substrate volatile solid added to the bottle (g)
Inoculum (negative control)	1800	-	7,37	-	-
Chicken manure	1612,28	187,72	21,10	3	39,60
Cow manure	1536,73	263,27	14,34	3	37,75
Chopped maize straw	1752,83	47,17	91,3	3	43,07
Starch (positive control)	1755,94	44,06	97,9	3	43,13

**TABLE 5: Weight (gram) values of the wastes used in the first experiment that should be added according to I/S = 3**

The weight (gram) values of the wastes used in the second experiment that should be added according to I/S = 4 were calculated as shown in Table 6.

	Amount of inoculum (g)	Amount of substrate (g)	%Volatile Solid	I/S	Total amount of substrate volatile solid added to the bottle (g)
Inoculum (negative control)	1800	-	4,35	-	-
Vinasse 17 brix	1655,93	144,07	12,5	4	18,01
Starch (positive control)	1780,62	19,38	99,9	4	19,36

**TABLE 6: Weight (gram) values of the wastes used in the second experiment that should be added according to I/S = 4**

The weight (gram) values of the wastes used in the third experiment, which should be added according to I/S = 4, were calculated as shown in Table 7.

	Amount of inoculum (g)	Amount of substrate (g)	%Volatile solid	I/S	Total amount of substrate volatile solid added to the bottle (g)
Inoculum (negative control)	1,800	-	4.28	-	-
Beet tail	1,687.17	112.83	16	4	18.05
Starch (positive control)	1,776.25	23.75	80	4	19.00

**TABLE 7: Weight (gram) values of the wastes used in the third experiment that should be added according to I/S = 4**

## Results

### Formula for calculation of BMP

The biomethane gas produced by the anaerobic breakdown of the substrate must be continuously monitored until the gas production has nearly ceased in order to determine the actual BMP of an organic material. Regulating the two flow rates and generating a report are the ideal ways to confirm this [7].

The BMP is the amount of methane produced per unit amount of organic substrate material supplied to the reactor. Consequently, the amount of organic substrate material introduced to the reactor must be divided by the total volume of the experiment. The volume fraction of biomethane in the experimental sample bottle must be subtracted from the total volume of accumulated biomethanes in order to calculate the actual production from the substrate. This is owing to the fact that the organic residues in the injected inoculum will also generate a certain quantity of biogas throughout the anaerobic process [8].

The volume of gas produced per gram of VS injected is the most interesting factor to consider when evaluating a substrate's biochemical methane potential (BMP).

When determining the gas output from the inoculum, use the blank (Equation 5).

$$BMP = \frac{V_S - V_B \times \left( \frac{m_{VS,IS}}{m_{VS,IB}} \right)}{m_{VS,SS}} \quad (5)$$

Parameter	Unit	Symbol
Accumulated biomethane volume from sample bottle(s)	Nml	□□
Accumulated biomethane volume from blank bottle(s)	Nml	□□
VS amount of substrate in sample bottle(s)	g	$m_{VS,SS}$
VS amount of inoculum in sample bottle(s)	g	$m_{VS,IS}$
VS amount of inoculum in blank bottle(s)	g	$m_{VS,IB}$

**TABLE 8: BMP parameters and properties**

BMP, Biochemical Methane Potential

Where

BMP - is the normalized volume of methane produced per gram VS of substrate added (NmlCH<sub>4</sub>/gVS)

Nml - is normalized milliliter

An example BMP calculation for chicken manure can be done as follows (Table 5):

if we substitute all the data in Equation (5),

BMP = 260,85 (NmlCH<sub>4</sub>/gVS) for chicken manure.

By substituting the parameters explained for BMP in Table 8 into Equation (5), the amount of biogas produced during the process was found to be 260.83 Nml.

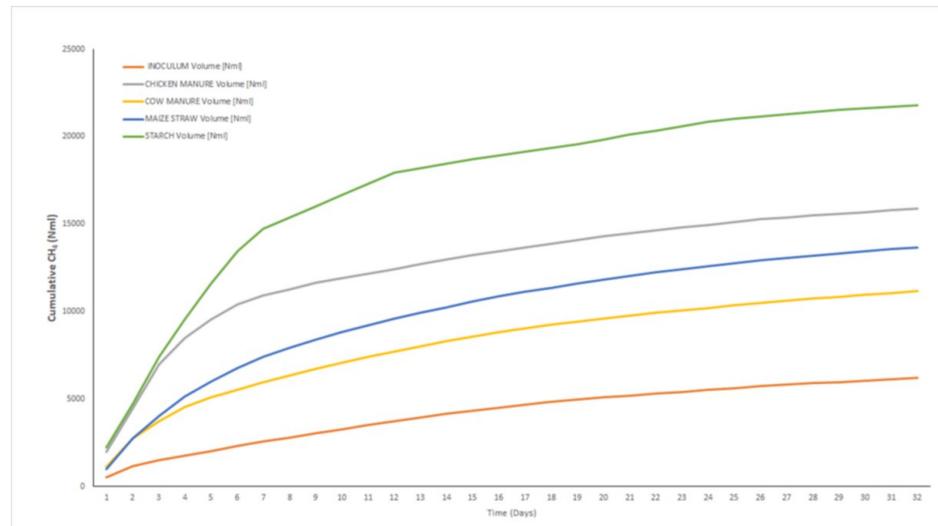
The obtained BMP values, together with the inoculum/substrate ratio value of 3, are listed in Table 9.

	Accumulated volume methane (ml)	BMP (NmICH <sub>4</sub> /gVS )
Inoculum (negative control)	6,192.89	-
Chicken manure	15,876.00	260.83
Cow manure	11,179.20	156.08
Chopped maize straw	13,672.00	177.42
Starch (positive control)	21,813.00	363.15

**TABLE 9: BMP values of the wastes used in the first experiment I/S = 3**

BMP, Biochemical Methane Potential

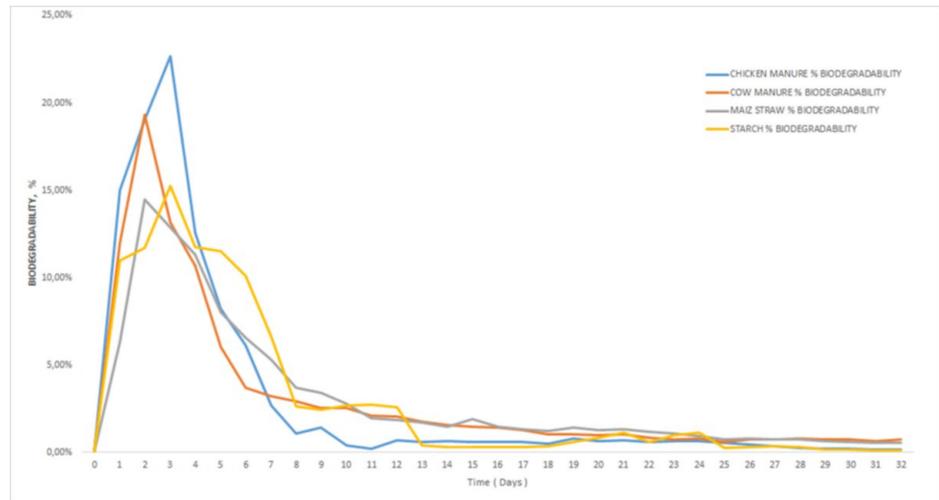
Figure 2 shows the methane gas values released when the I/S of inoculum, chicken manure, cow manure, corn straw, and starch are 3, in order to compare the methane yields.



**FIGURE 2: Cumulative methane yield of inoculum, chicken manure, cow manure, maize straw, and starch @ I/S = 3**

In parallel with the examination of the methane yields of the wastes in Figure 2, the biodegradability rates of these materials at I/S:3 are given in Table 3. Biodegradability is directly related to methane yield.

Figure 3 shows that the biodegradation rates for chicken manure were 89.17% in the first 10 days, cow manure 76.15%, maize straw 74.63%, and starch 85.67%, with an average degradation rate of 81.41% for the first 10 days.



**FIGURE 3: Biodegradability rates (%) of chicken manure, cow manure, maize straw, and starch @ I/S = 3**

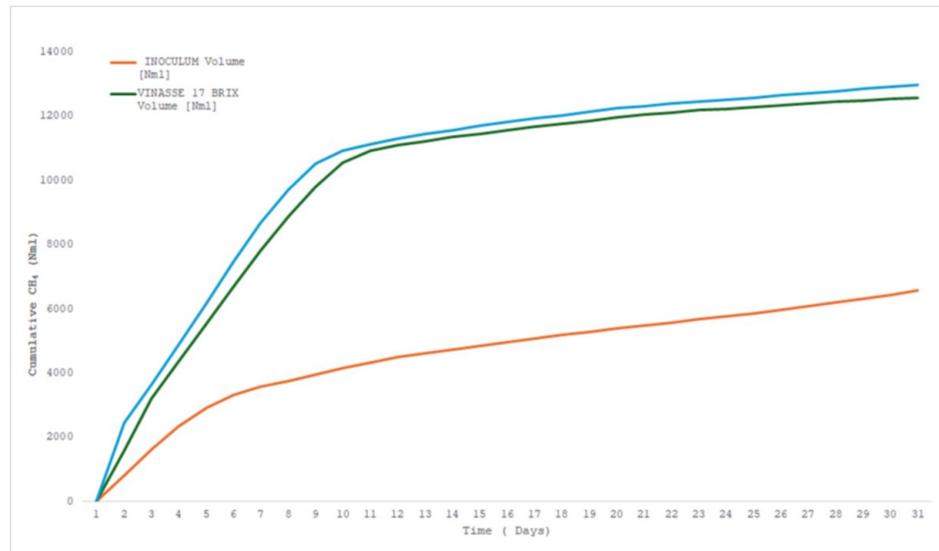
In the second experiment in the study, when the inoculum/substrate ratio (I/S) is 4, the BMP value of organic wastes is as shown in Table 10.

	Accumulated volume methane (ml)	BMP (NmICH <sub>4</sub> /gVS)
Inoculum (negative control)	6,569.7	-
Vinasse 17 brix	12,753.9	372.57
Starch (positive control)	12,981.1	334.82

**TABLE 10: BMP values of the wastes used in the second experiment I/S = 4**

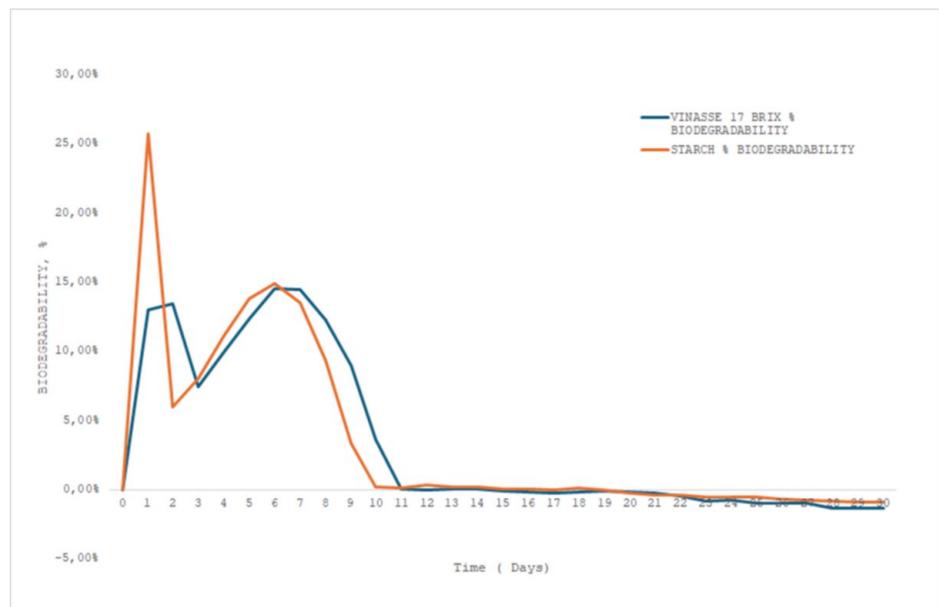
BMP, Biochemical Methane Potential

In the case where I/S is 4, the methane gas formation process in the 39-day period is shown in Figure 4.



**FIGURE 4: Cumulative methane yield of inoculum, vinasse 17 brix and starch @ I/S = 4**

When I/S is 4, the biodegradability of the organic wastes whose methane gas formation is monitored is shown in Figure 5.



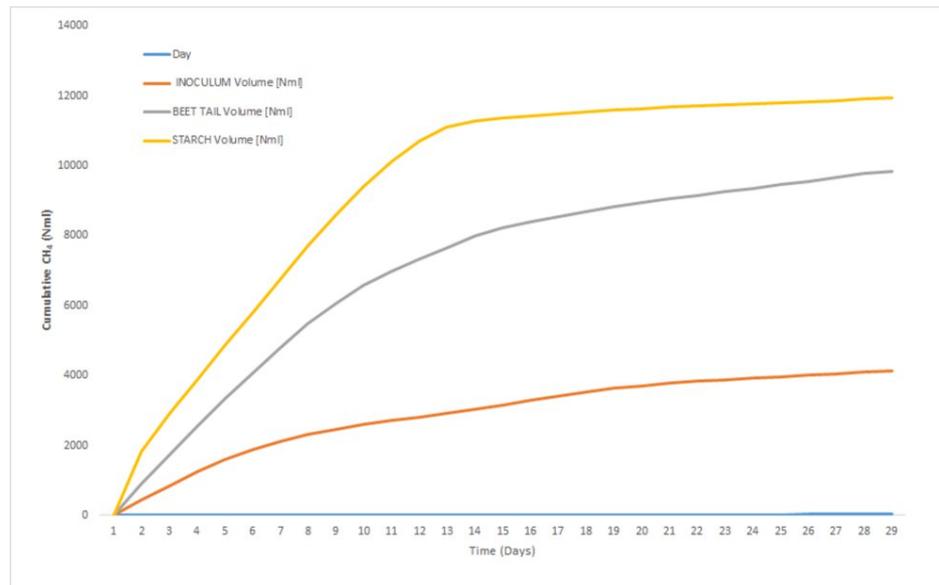
**FIGURE 5: Biodegradability rates (%) of vinasse 17 brix and starch @ I/S = 4**

The methane gas output values of the study designed as the third experiment and including beet tail, inoculum, starch under the conditions of I/S: 4 are as shown in Table 11.

	Accumulated volume methane (Nml)	BMP (NmICH <sub>4</sub> /gVS)
Inoculum (negative control)	4,130.8	-
Beet tail	9,850.1	331.19
Starch (positive control)	11,944	331.28

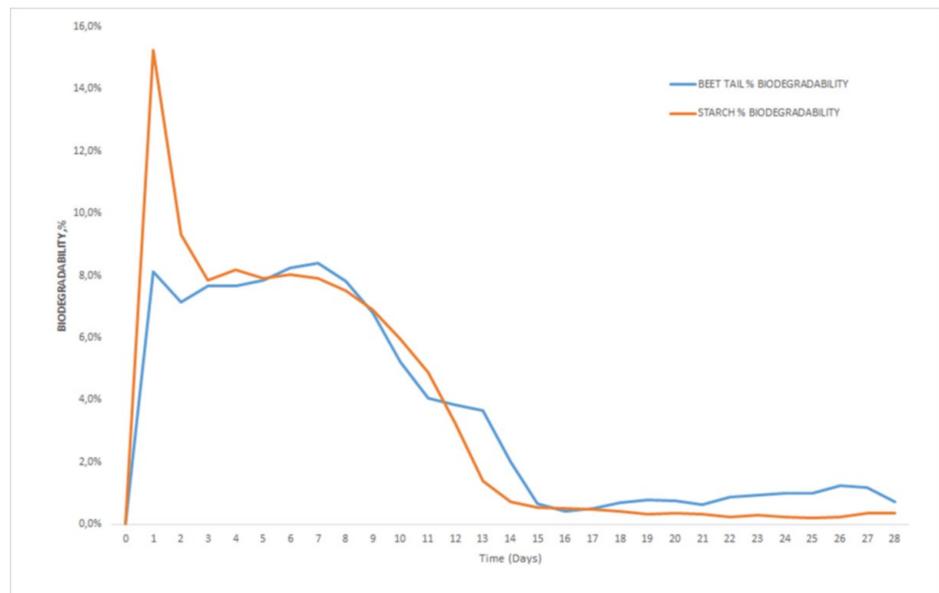
**TABLE 11: BMP values of the wastes used in the third experiment I/S = 4**

BMP, Biochemical Methane Potential



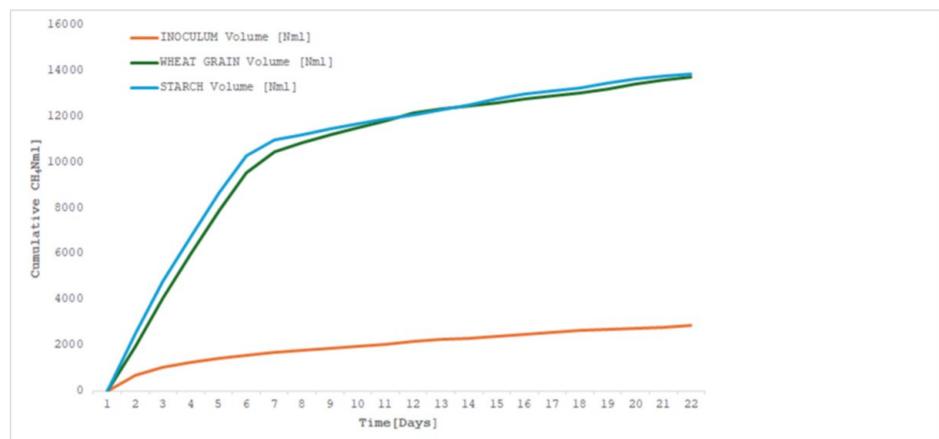
**FIGURE 6: Cumulative methane yield of inoculum, beet tail, and starch @ I/S = 4**

The 39-day methane gas output process of the organic wastes whose methane output value is shown in Table 11 is as shown in Figure 6.



**FIGURE 7: Biodegradability rates (%) of beet tail and starch @ I/S = 4**

In parallel with this, the biodegradability of these organic wastes in the 39-day period is shown in Figure 7.



**FIGURE 8: Cumulative methane yield of wheat grain and (wheat) starch @ I/S = 2**

The methane yields and graphs of wheat grain and wheat starch, and consequently their rates of degradation, are identical in a different experiment with I/S = 2 (Figure 8). Consequently, wheat starch will be utilized in place of wheat grain when calculating electricity.

As previously stated, the literature revealed a starch methane output of  $350 \pm 33$ . One may say that the first trial's positive control was correctly implemented, and the analysis results were appropriately prepared using I/S ratios.

### Methane content in total biogas and kWh calculation from 1 m<sup>3</sup> of methane

The amounts of biogas burned in gas engines and the corresponding amounts of methane were calculated at Meram Renewable Energy Production Inc. (01.02.2023-28.02.2023, Table 12).

Based on the monthly consumed biogas amounts, facility operating hours and gross energy produced data, 216,001 kWh of energy was produced corresponding to 103,355 m<sup>3</sup> of biogas (Table 13).

Total consumed methane value (average 57.47%) between the specified dates is 60,032.9 m<sup>3</sup>. If the total amount of energy produced in a month is 216,001 kWh, to find how many kWh is 1 m<sup>3</sup> of methane:

$$216,001/60,032.9 = 3.60 \text{ kWh}/1 \text{ m}^3 \text{ methane}$$

Date	Total biogas consumed by engines, m <sup>3</sup>	Produced kWh/day	Working hours	Consumed biogas m <sup>3</sup> /MWh	% Methane content in biogas	Consumed methane m <sup>3</sup> /MWh	Total consumed methane	1 m <sup>3</sup> CH <sub>4</sub> to kWh	Produced kWh
1.02.2023	16,541.0	34,582.2	23.6	478,3	57,7	276,0	9.544,2	3,6	1.464,6
2.02.2023	6,985.0	14,587.7	10.4	478,8	58,0	277,7	4.051,3	3,6	1.405,7
3.02.2023	201.0	391.2	0.4	513,8	54,5	280,0	109,5	3,6	1.113,5
4.02.2023	3,589.0	7,250.0	5.1	495,0	56,1	277,7	2.013,4	3,6	1.421,6
5.02.2023	4,050.0	8,485.9	6.0	477,3	58,3	278,2	2.361,2	3,6	1.407,3
6.02.2023	2,987.0	6,342.1	4.9	471,0	59,5	280,2	1.777,3	3,6	1.299,5
7.02.2023	2,040.0	4,209.0	3.2	484,7	57,7	279,7	1.177,1	3,6	1.298,7
8.02.2023	1,800.0	3,720.3	2.6	483,8	57,8	279,7	1.040,4	3,6	1.424,4
9.02.2023	285.0	600.0	0.4	475,0	58,4	277,4	166,4	3,6	1.500,0
10.02.2023	360.0	758.2	0.6	474,8	58,5	277,8	210,6	3,6	1.180,8
11.02.2023	1,710.0	3,407.4	2.4	501,9	55,6	279,0	950,6	3,6	1.408,4
12.02.2023	1,270.0	2,562.4	1.8	495,6	56,4	279,5	716,3	3,6	1.459,1
13.02.2023	282.0	581.2	0.5	485,2	57,2	277,5	161,3	3,6	1.251,5
14.02.2023	1,330.0	2,750.0	2.0	483,6	57,4	277,6	763,4	3,6	1.375,0
15.02.2023	1,240.0	2,562.0	1.8	484,0	57,5	278,3	713,0	3,6	1.423,3
16.02.2023	1,940.0	4,000.0	3.0	485,0	57,5	278,9	1.115,5	3,6	1.333,3
17.02.2023	4,150.0	8,486.0	6.0	489,0	56,5	276,3	2.344,8	3,6	1.414,3
18.02.2023	0.0	0.0	0.0	0.0	56,4	0.0	0.0	0.0	0.0
19.02.2023	0.0	0.0	0.0	0.0	56,0	0.0	0.0	0.0	0.0
20.02.2023	0.0	0.0	0.0	0.0	55,9	0.0	0.0	0.0	0.0
21.02.2023	0.0	0.0	0.0	0.0	57,4	0.0	0.0	0.0	0.0
22.02.2023	0.0	0.0	0.0	0.0	58,1	0.0	0.0	0.0	0.0
23.02.2023	8,050.0	17,061.5	11.7	471,8	59,0	278,4	4.749,5	3,6	1.454,7
24.02.2023	16,985.0	35,886.5	23.9	473,3	58,9	278,8	10.004,2	3,6	1.501,3
25.02.2023	13,500.0	28,424.9	19.0	474,9	58,6	278,3	7.909,7	3,6	1.498,0
26.02.2023	2,450.0	5,180.6	3.8	472,9	58,6	277,1	1.435,5	3,6	1.362,4
27.02.2023	6,350.0	13,261.3	9.2	478,8	58,1	278,2	3.688,7	3,6	1.438,3
28.02.2023	5,260.0	10,910.2	7.7	482,1	57,6	277,7	3.029,2	3,6	1.409,4

**TABLE 12: One month of biogas and electricity produced amounts, real factory data**

Total biogas consumed by engines, m <sup>3</sup>	Methane, %	Total methane consumed by engines, m <sup>3</sup>	Total produced electricity, kWh	How much kWh of electricity is produced with 1 m <sup>3</sup> of methane? kWh
103,355	57.47	60,032.9	216,001	3.6

**TABLE 13: The calculation of kWh of electricity produced in 1 month is how many kWh of electricity can be produced from 1 m<sup>3</sup> CH<sub>4</sub>?**

Based on these tables, it can be said that 3.6 kWh electricity will be produced for 1 m<sup>3</sup> methane. Since the laboratory results we found are over Nm<sup>3</sup>/g.VS, they will be entered as m<sup>3</sup>/ton.VS when converting units.

**Formulation for Calculation of Electricity from Fresh Manure**

$$E_{(kWh)} = \left( \frac{m_f \times \%DM \times \%ODM}{10000} \right) \times \frac{BMP_m}{1000} \times c \quad (6)$$

Where

$E_{(kWh)}$ : Electricity produced from electric motors (kWh),

$m_f$ : Fresh manure amount, kg,

%DM: Dry matter of the manure,

%ODM: Organic dry matter of the manure,

BMP<sub>m</sub>: Biochemical methane potential of the manure, Nm<sup>3</sup>CH<sub>4</sub>/g.VS

c: methane conversion factor, 3.60 (1 m<sup>3</sup> CH<sub>4</sub> = 3.6 kWh, from Table 12)

10000: % conversion factor of fresh manure to % volatile solid

1000: conversion factor of g to kg.

(%DMX%ODM/100 = %VS for calculation of %VS)

Example calculation for chicken manure (Table 14):

Total amount, kg	%Dry matter	%Organic dry matter	1 m <sup>3</sup> CH <sub>4</sub> to kWh	BMP <sub>m</sub>	E <sub>kWh</sub>
$m_f$	%DM	%ODM	c	BMP for Chicken manure	kWh
1000	33.99	62.09	3.6	260.83	198.17

**TABLE 14: Chicken manure BMP example calculation data**

BMP, Biochemical Methane Potential

If we substitute the values in Table 13 into Equation (6):

kWh is found. In other words, since 1000 kWh = 1 MWh, 1000/198.17 = 5.05 tons of fresh chicken manure is needed to produce 1 MWh of electricity. From this, we can calculate how much electricity they will generate per ton for each raw material (Table 15).

Raw materials	%DM	%ODM	BMP (Nm <sup>3</sup> CH <sub>4</sub> /gVS)	Amount of energy that can be obtained from 1 Ton raw material (kWh)	Amount (ton) of raw materials required for 1 MWh energy production
Chicken manure	33.99	62.09	260.83	198.17	5.05
Cow manure	17.53	81.83	156.08	80.60	12.41
Powdered maize straw	96	95.1	177.42	583.12	1.71
Starch (positive control), (wheat grain)	98	99.9	363.15	1,279.91	0.78
Vinasse 17 brix	17.1	73.1	372.57	167.66	5.96
Beet tail	16.8	95.24	331.19	190.77	5.24

**TABLE 15: How much electricity will be generated per ton from the raw materials used**

When chopped straw was used in this study, the analyses were performed on dry material, but the biogas was fed to the digesters at 25% dry matter and 18% VS content.

The biological degradation rates of the organic materials in the experimental process in the 28-day period within the study are shown in Table 16.

Day	Chicken manure % biodegradability	Cow manure % biodegradability	Maize straw % biodegradability	Starch (wheat grain) % biodegradability	Vinasse 17 Brix % biodegradability*	Beet tail % biodegradability
	A	B	C	D	E	F
1	14,98%	11,99%	6,32%	11,00%	13,02%	8,14%
2	19,05%	19,33%	14,46%	11,69%	13,46%	7,15%
3	22,66%	13,16%	12,85%	15,23%	7,40%	7,67%
4	12,59%	10,70%	11,30%	11,76%	9,91%	7,67%
5	8,21%	6,04%	8,00%	11,53%	12,36%	7,85%
6	6,12%	3,72%	6,55%	10,10%	14,57%	8,26%
7	2,67%	3,23%	5,31%	6,59%	14,48%	8,40%
8	1,08%	2,94%	3,68%	2,65%	12,27%	7,81%
9	1,40%	2,52%	3,39%	2,43%	9,00%	6,82%
10	0,40%	2,52%	2,76%	2,69%	3,63%	5,22%
11	0,19%	2,08%	1,97%	2,76%	0,03%	4,05%
12	0,70%	2,06%	1,86%	2,58%	-0,01%	3,85%
13	0,60%	1,74%	1,72%	0,41%	0,02%	3,65%
14	0,63%	1,58%	1,46%	0,31%	0,07%	2,00%
15	0,60%	1,46%	1,89%	0,30%	-0,12%	0,65%
16	0,59%	1,41%	1,49%	0,30%	-0,18%	0,42%
17	0,60%	1,27%	1,30%	0,31%	-0,25%	0,53%
18	0,51%	1,03%	1,24%	0,37%	-0,17%	0,69%
19	0,77%	1,01%	1,40%	0,58%	-0,11%	0,80%
20	0,67%	0,98%	1,27%	0,83%	-0,17%	0,76%
21	0,67%	1,02%	1,30%	1,13%	-0,26%	0,65%
22	0,59%	0,85%	1,16%	0,60%	-0,44%	0,88%
23	0,65%	0,76%	1,07%	1,00%	-0,82%	0,93%
24	0,63%	0,79%	0,94%	1,11%	-0,74%	1,01%
25	0,54%	0,62%	0,74%	0,27%	-0,97%	0,99%
26	0,47%	0,77%	0,77%	0,29%	-0,96%	1,23%
27	0,37%	0,77%	0,75%	0,33%	-1,01%	1,18%
28	0,27%	0,79%	0,72%	0,30%	-1,32%	0,73%

**TABLE 16: Biodegradability of raw materials for 28 days**

$$EA_{28} = (A \times i_{28} + A \times i_{27} + \dots + A \times i_1) xc(7)$$

$$EA_1 = (A \times i_1) xc(8)$$

Where

$EA_{28}$  is total amount of electricity supplied by fed chicken manure for 1 month, kWh,

A is total methane yield of fed chicken manure after 1 month,  $m^3$ ,

c is the conversion factor (3.6) of 1 m<sup>3</sup> methane to kWh,

i<sub>1</sub> to i<sub>28</sub> is from day 1 to day 28 % biodegradability rates (Table 16).

Example calculation of chicken manure:

152,060 kg of chicken manure:

$$A = 8,370.38 \text{ m}^3 \text{ CH}_4$$

To find the total electrical energy on day 5; (from Equation 7)

$$EA_5 = (A \times i_5 + A \times i_4 + \dots + A \times i_1) \times c$$

If we find EA<sub>28</sub> (total electric, kWh)

$$EA_{28} = (A \times i_{28} + A \times i_{27} + \dots + A \times i_1) \times c$$

It is possible to see from the formulation that 23,351 kWh of the total electricity potential of 30,134 kWh was received in the first 5 days.

EA for chicken manure, EB for cow manure, EC or maize straw, ED for wheat grain (starch), EE for vinasse 17 brix, EF for beet tail notations.

The feeding amounts of each organic material as a reflection of the real factory values are shown in Table 17.

Total feed in biogas (Kg)						
Date	Chicken manure	Cow manure	Maize straw	Starch	Vinasse	Beet tail
1.01.2023	152.060	154.785	0	12.532	12.469	5.210
2.01.2023	115.400	145.983	0	11.254	13.454	4.294
3.01.2023	102.546	147.440	23.800	5.875	16.741	3.867
4.01.2023	124.621	154.297	52.880	3.625	15.421	4.621
5.01.2023	114.624	162.459	46.680	5.321	0	3.978
6.01.2023	137.420	145.753	0	9.654	0	4.651
7.01.2023	135.951	142.512	0	0	9.624	4.357
8.01.2023	85.200	146.214	9.880	0	15.467	4.952
9.01.2023	43.500	132.254	0	7.512	16.421	4.935
10.01.2023	98.952	108.200	0	6.431	12.465	4.261
11.01.2023	115	103.040	0	4.972	13.451	4.953
12.01.2023	112.475	50.480	6.618	5.641	0	4.324
13.01.2023	132.751	163.742	6.174	0	0	5.124
14.01.2023	145.632	159.480	5.326	0	0	0
15.01.2023	154.357	182.600	6.014	4.697	17.646	0
16.01.2023	142.162	191.420	4.712	5.431	16.421	0
17.01.2023	132.495	191.564	0	9.734	16.723	0
18.01.2023	142.657	177.000	0	0	15.431	4.621
19.01.2023	165.349	145.000	0	27.660	17.534	4.532
20.01.2023	142.159	165.920	0	31.960	0	4.987

21.01.2023	147.528	144.300	0	28.540	0	4.589
22.01.2023	168.520	262.560	0	24.040	16.598	4.561
23.01.2023	298.020	245.680	0	21.440	15.425	4.523
24.01.2023	102.380	254.300	0	17.100	16.421	0
25.01.2023	216.100	311.240	0	20.900	14.741	0
26.01.2023	172.300	57.240	0	0	14.531	4.213
27.01.2023	232.640	209.980	0	0	15.643	4.698
28.01.2023	261.100	191.760	0	22.860	12.367	5.013
29.01.2023	175.380	404.360	0	22.640	0	4.853
30.01.2023	183.160	347.320	0	21.700	0	4.532
31.01.2023	195.632	324.621	0	0	0	5.321
1.02.2023	185.462	214.632	0	0	15.643	4.256
2.02.2023	210.695	165.874	13.780	31.600	15.987	4.879
3.02.2023	304.562	256.412	17.652	39.240	14.563	4.632
4.02.2023	301.265	145.698	0	0	13.245	0
5.02.2023	298.752	0	0	0	12.498	0
6.02.2023	254.621	46.820	0	23.800	12.245	0
7.02.2023	214.587	91.880	7.563	16.460	15.423	4.675
8.02.2023	234.951	0	0	0	0	4.215
9.02.2023	145.236	0	0	0	0	4.634
10.02.2023	356.960	159.632	46.160	27.860	0	4.125
11.02.2023	189.440	175.278	62.820	23.800	17.451	4.532
12.02.2023	94.300	0	56.540	18.160	15.456	4.123
13.02.2023	248.280	61.320	37.000	22.860	14.987	5.698
14.02.2023	334.800	108.300	20.000	9.764	16.561	4.561
15.02.2023	121.875	145.832	0	9.612	16.498	4.951
16.02.2023	231.924	187.356	0	8.531	16.541	0
17.02.2023	261.900	337.160	0	18.280	15.951	0
18.02.2023	214.276	0	0	17.920	0	0
19.02.2023	271.740	0	0	19.500	0	4.216
20.02.2023	283.400	90.960	0	0	0	4.124
21.02.2023	233.000	127.500	0	8.220	14.623	4.863
22.02.2023	209.660	236.920	0	0	16.547	4.721
23.02.2023	285.260	124.860	0	6.160	16.527	4.632
24.02.2023	262.640	206.720	6.466	5.760	14.731	4.698
25.02.2023	158.300	256.320	6.262	12.560	16.534	0
26.02.2023	275.840	282.540	3.970	9.660	15.321	0
27.02.2023	172.220	217.860	36.860	10.980	13.245	0
28.02.2023	230.140	139.720	58.640	11.180	15.123	5.621

TABLE 17: Total feed in biogas for 2 months

## Discussion

This study successfully determined the BMP of various feedstocks commonly used in biogas production under mesophilic laboratory conditions. The key raw materials analyzed included chicken manure, cow manure, chopped maize straw, vinasse (17 brix), beet tail, and wheat grain (represented by starch). The BMP values obtained were 260.83 NmlCH<sub>4</sub>/gVS for chicken manure, 156.08 NmlCH<sub>4</sub>/gVS for cow manure, 177.42 NmlCH<sub>4</sub>/gVS for chopped maize straw, 372.57 NmlCH<sub>4</sub>/gVS for vinasse 17 brix, and 331.19 NmlCH<sub>4</sub>/gVS for beet tail. Wheat starch, used as a positive control and representing wheat grain, yielded BMP values around 331-363 NmlCH<sub>4</sub>/gVS across different experiments, aligning reasonably well with literature values (350 ± 33 NmlCH<sub>4</sub>/gVS), validating the experimental setup and methodology.

A significant aspect of this research was the comparison between laboratory-derived BMP data and actual energy production in a real biogas reactor. By analyzing the daily degradation rates of each feedstock over a 28-day period (mirroring the typical hydraulic retention time in real digesters) and using real factory feed data for a 1-month period, the study demonstrated a strong correlation. The methodology involved calculating the expected electricity generation (kWh) based on the measured BMP values, feedstock characteristics (%DM, %VS), daily feed amounts, and a calculated conversion factor of 3.60 kWh per m<sup>3</sup> of methane derived from plant operational data.

The comparison revealed that the electricity generation predicted using the laboratory BMP data and degradation rates achieved approximately 94% accuracy compared to the actual electricity produced by the biogas plant over the reference month. The expected output was calculated as 2,250,640 kWh, while the actual recorded output was 2,398,126 kWh. This high degree of accuracy underscores the reliability of laboratory BMP tests, when combined with detailed degradation analysis, for estimating the energy potential of feedstocks in full-scale biogas operations.

The study highlights the practical value of conducting thorough BMP analysis prior to establishing or modifying biogas operations. Relying solely on theoretical data can be misleading; determining the specific methane potential and degradation profile of available raw materials provides a much more accurate basis for feasibility studies and operational planning. The detailed daily biodegradability data presented (Table 15) further enhances the understanding of how different feedstocks contribute to gas production over time, noting, for instance, the rapid initial degradation of vinasse compared to other substrates.

Methodologically, the study employed standard procedures, including the characterization of feedstocks for TS and VS, the use of an AMPTS II, and CO<sub>2</sub> scrubbing with NaOH. The use of inoculum from an active digester and maintaining appropriate I/S ratios (primarily 3 or 4) ensured stable anaerobic digestion conditions.

In the experiment conducted over 2 months of real factory data covering the dates 01.01.2023-28.02.203, however, in order to make sense of the data in the created system, one-month data dated 01.02.2024-28.02.2024 was taken as the reference. 6.586.086 kg chicken manure, 3.779.594 kg cow manure, 373.713 kg maize straw (25% DM), 351.907 kg wheat grain, 335.700 kg vinasse 17 brix, 88.156 kg beet tail were fed (Table 18).

	Chicken manure	Cow manure	Maize straw	Wheat grain	Vinasse 17 Birc	Beet tail
Amount (kg)	6,586,086.0	6,586,086.0	373,713.0	351,907.0	335,700.0	88,156.0
BMP (NmL/g.VS)	260.8	156.1	177.4	363.2	372.6	331.2
DM (%)	34.0	17.5	25.0	98.0	17.1	16.8
ODM (%)	62.1	81.8	72.0	99.0	73.1	95.2
E <sub>kWh</sub> (kWh)	1,305,149.5	530,849.8	42,965.1	446,352.3	56,282.7	16,817.0
E <sub>kWh</sub> (kWh) Total	2,398,416.8					

**TABLE 18: Total feeds between 01.02.2024 and 28.02.2024**

BMP, Biochemical Methane Potential; DM, dry matter; ODM, organic dry matter

The electricity generated as a result of these feedings for 1 month (real factory report) was recorded as 2.398.126 kWh. The expected electricity production from the detailed calculation we created from the BMP data found by laboratory analyzes is 2.250.640 kWh. With an approach close to 94%, it was found how much electricity would be produced.

Month 1	Expected production according to BMP analysis kWh	Actual factory data kWh	Realization %	Month 2	Expected production according to BMP analysis kWh	Actual factory data kWh	Realization %
1.01.2023	8,425.6	65,525	13%	1.02.2023	78,686.1	81,794	96%
2.01.2023	17,828.4	66,504	27%	2.02.2023	79,466.6	82,147	97%
3.01.2023	26,706.3	66,103	40%	3.02.2023	85,479.9	90,690	94%
4.01.2023	32,027.7	66,760	48%	4.02.2023	87,606.6	90,179	97%
5.01.2023	35,890.6	69,273	52%	5.02.2023	87,063.1	89,189	98%
6.01.2023	40,136.6	70,808	57%	6.02.2023	85,300.8	87,587	97%
7.01.2023	41,069	70,633	58%	7.02.2023	85,279.3	89,530	95%
8.01.2023	40,058.7	67,953	59%	8.02.2023	81,501	87,794	93%
9.01.2023	37,549.8	63,241	59%	9.02.2023	70,858.8	72,700	97%
10.01.2023	35,529.7	57,613	62%	10.02.2023	74,687	84,935	88%
11.01.2023	35,098.8	58,309	60%	11.02.2023	78,808.1	88,210	89%
12.01.2023	35,600.7	62,823	57%	12.02.2023	79,562.2	89,420	89%
13.01.2023	36,254.4	66,367	55%	13.02.2023	77,271.9	84,706	91%
14.01.2023	38,820	67,807	57%	14.02.2023	79,051.9	83,660	94%
15.01.2023	42,393.9	66,770	63%	15.02.2023	79,923.3	82,808	97%
16.01.2023	45,185.1	67,021	67%	16.02.2023	80,391.7	82,840	97%
17.01.2023	47,086.2	62,775	75%	17.02.2023	81,300.8	84,295	96%
18.01.2023	47,005.6	66,063	71%	18.02.2023	82,217.5	84,311	98%
19.01.2023	51,662.1	67,391	77%	19.02.2023	81,224.2	85,502	95%
20.01.2023	52,689	75,683	70%	20.02.2023	78,787.8	87,588	90%
21.01.2023	57,913.8	74,772	77%	21.02.2023	80,401.1	88,306	91%
22.01.2023	61,859	79,357	78%	22.02.2023	78,415.3	88,627	88%
23.01.2023	72,264.3	82,951	87%	23.02.2023	77,858.3	87,074	89%
24.01.2023	74,885.8	90,430	83%	24.02.2023	76,950.2	85,406	90%
25.01.2023	80,263.2	89,334	90%	25.02.2023	77,185.6	85,922	90%
26.01.2023	74,548.5	87,200	85%	26.02.2023	82,175.1	85,163	96%
27.01.2023	73,727.5	83,133	89%	27.02.2023	81,579.7	83,895	97%
28.01.2023	76,122.1	78,746	97%	28.02.2023	81,606.3	83,848	97%
29.01.2023	78,762.1	85,576	92%				
30.01.2023	83,608.7	86,678	96%				
31.01.2023	81,093.6	86,862	93%				

**TABLE 19: Real factory feedings**

The actual factory report and the calculation from the BMP results are almost identical. However, two months of data were analyzed during the study and the trends of the last month were taken into account in order for the system to work fully in the first days and the calculations to hold (Table 19). In the first month, the first month data was ignored because there were biodegradation from previous months.

In order to be able to make statistical analysis, the BMP methane gas values obtained in the study are also shown graphically as a whole. Since similar results were obtained in multiple data analysis for the same I/S ratio during the experiments, they were included in the article as average values.

The fact that vinasse, a waste of the sugar production industry, provides high methane production (12,753.9 ml) with a dry matter content of 14% and that starch, which is richer in terms of organic content than vinasse (334.82 Nml CH<sub>4</sub>/gVS), provides an energy production value close to vinasse (372.57 Nml CH<sub>4</sub>/gVS) in biogas production is an important alternative presented to the producer in terms of circular economy applications in terms of its evaluation on an industrial scale.

One of the interesting applications of the circular economy would be the large amount of vinasse (>1 ton/day) produced as sugar industry waste and the producer prefers to use it for energy production rather than disposal. In this respect, when the raw material needs in energy production are taken into consideration, the fact that the commercial value of vinasse is lower than that of raw materials such as coal and lignite used in energy production will provide a very economical alternative to the producer, thus, when it comes to energy production, the sensitivity level in supplying vinasse and guaranteeing its dry matter content will be lower than other energy raw materials.

## Conclusions

BMP analyses were compared with real facility data and a successful electricity production estimate of 94% was obtained. When the organic and dry materials of the raw materials taken to the factory were carefully examined, it was seen that it was possible to reach real electricity production values with the decomposition rates we obtained in the BMP analysis. Newly established biogas companies should not act only with theoretical data, it is useful to determine the capacity after determining the methane potential of the raw material. In the experiment conducted with the two-month real factory data subject to the study, 6,586,086 kg of chicken manure, 3,779,594 kg of cow manure, 373,713 kg of corn straw (%25 DM), 351,907 kg of wheat grain, 335,700 kg of vinasse 17 brix, 88,156 kg of beet tail were given as feed. As a result of these feeds, the electricity produced for a month was recorded as 2,398,126 kWh. Electricity production generated from BMP data found through laboratory analysis will produce 2,250,640 kWh of electrical energy with 94% efficiency.

## Additional Information

### Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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### Disclosures

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**Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue.

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## References

1. Odejobi OJ, Ajala OO, Osuolale FN: Review on potential of using agricultural, municipal solid and industrial wastes as substrates for biogas production in Nigeria. *Biomass Conversion and Biorefinery*. 2024, 14:1567-79. [10.1007/s13399-022-02613-y](https://doi.org/10.1007/s13399-022-02613-y)
2. Manyi-Loh CE, Mamphweli SN, Meyer E, Okoh A, Makaka G, Simon M: Microbial anaerobic digestion (biogas) as an approach to the decontamination of animal wastes in pollution control and the generation of renewable energy. *International Journal of Environmental Research and Public Health*. 2013, 10:4390-417.

- [10.3390/ijerph10094390](https://doi.org/10.3390/ijerph10094390)
3. Sarker S, Lamb JJ, Hjelme DR, Lien KM: A review of the role of critical parameters in the design and operation of biogas production plants. *Applied Sciences*. 2019, 9:1915. [10.3390/app9091915](https://doi.org/10.3390/app9091915)
  4. Chulalaksananukul S, Sinbuathong N, Chulalaksananukul W: Bioconversion of pineapple solid waste under anaerobic condition through biogas production. *KKU Research Journal*. 2012, 17:734-42.
  5. Velmurugan B, Ramanujam RA: Anaerobic digestion of vegetable wastes for biogas production in a fed-batch reactor. *International Journal of Emerging Sciences*. 2011, 1:478.
  6. Lin J, Zuo J, Gan L, et al.: Effects of mixture ratio on anaerobic co-digestion with fruit and vegetable waste and food waste of China. *Journal of Environmental Sciences*. 2011, 23:1403-408. [10.1016/s1001-0742\(10\)60572-4](https://doi.org/10.1016/s1001-0742(10)60572-4)
  7. Amon T, Amon B, Kryvoruchko V, Zollitsch W, Mayer K, Gruber L: Biogas production from maize and dairy cattle manure—Influence of biomass composition on the methane yield. *Agriculture Ecosystems & Environment*. 2007, 118:173-82. [10.1016/j.agee.2006.05.007](https://doi.org/10.1016/j.agee.2006.05.007)
  8. Li Y, Zhang R, Chen C, Liu G, He Y, Liu X: Biogas production from co-digestion of corn stover and chicken manure under anaerobic wet, hemi-solid, and solid state conditions. *Bioresource Technology*. 2013, 149:406-12. [10.1016/j.biortech.2013.09.091](https://doi.org/10.1016/j.biortech.2013.09.091)
  9. Kafle GK, Park JT, Kim SH, Ki S: Biogas production from the mixture of Chinese cabbage silage and swine manure. *Journal of Agricultural, Life and Environmental Sciences*. 2011, 23:43-46.
  10. Neo S, Vintilă T, Bura M: Conversion of agricultural wastes to biogas using as inoculum cattle manure and activated sludge. *Animal Science and Biotechnologies*. 2012, 1:328-34.
  11. Kabeyi MJB, Olanrewaju OA: Biogas production and applications in the sustainable energy transition. *Journal of Energy*. 2022, 2022:8750221. [10.1155/2022/8750221](https://doi.org/10.1155/2022/8750221)
  12. Samoraj M, Mironiuk M, Izydorczyk G, Witek-Krowiak A, Szopa D, Moustakas K, Chojnacka K: The challenges and perspectives for anaerobic digestion of animal waste and fertilizer application of the digestate. *Chemosphere*. 2022, 295:133799. [10.1016/j.chemosphere.2022.133799](https://doi.org/10.1016/j.chemosphere.2022.133799)
  13. Zhang Y, Li C, Yuan Z, Wang R, Angelidaki I, Zhu G: Syntrophy mechanism, microbial population, and process optimization for volatile fatty acids metabolism in anaerobic digestion. *Chemical Engineering Journal*. 2023, 452:139137. [10.1016/j.cej.2022.139137](https://doi.org/10.1016/j.cej.2022.139137)
  14. Feng Y, Lu J, Shen Z, et al.: Sequentially modified carbon felt for enhanced p-nitrophenol biodegradation through direct interspecific electron transfer. *Journal of Hazardous Materials*. 2023, 451:131055. [10.1016/j.jhazmat.2023.131055](https://doi.org/10.1016/j.jhazmat.2023.131055)
  15. Holewa-Rataj J, Rataj M, Kapusta P, Brzeszcz J, Janiga M, Król A: Home biogas production from organic waste: challenges and process optimization of methane fermentation. *Energies*. 2025, 18:1745. [10.3390/en18071745](https://doi.org/10.3390/en18071745)
  16. Xue S, Wang Y, Lyu X, Zhao N, Song J, Wang X, Yang G: Interactive effects of carbohydrate, lipid, protein composition and carbon/nitrogen ratio on biogas production of different food wastes. *Bioresource Technology*. 2020, 312:123566. [10.1016/j.biortech.2020.123566](https://doi.org/10.1016/j.biortech.2020.123566)
  17. Ahmadi-Pirlou M, Mesri Gundoshmian T: The effect of substrate ratio and total solids on biogas production from anaerobic co-digestion of municipal solid waste and sewage sludge. *Journal of Material Cycles and Waste Management*. 2021, 23:1938-946. [10.1007/s10163-021-01264-x](https://doi.org/10.1007/s10163-021-01264-x)
  18. Xu Q, Qiao Q, Gao Y, et al.: Gut microbiota and their role in health and metabolic disease of dairy cow. *Frontiers in nutrition*. 2021, 8:701511. [10.3389/fnut.2021.701511](https://doi.org/10.3389/fnut.2021.701511)
  19. Massé DI, Talbot G, Gilbert Y: On farm biogas production: A method to reduce GHG emissions and develop more sustainable livestock operations. *Animal Feed Science and Technology*. 2011, 166-167:436-45. [10.1016/j.anifeedsci.2011.04.075](https://doi.org/10.1016/j.anifeedsci.2011.04.075)
  20. Li R, Chen S, Li X: Anaerobic co-digestion of kitchen waste and cattle manure for methane production. *Energy Sources Part A Recovery Utilization and Environmental Effects*. 2009, 31:1848-856. [10.1080/15567030802606038](https://doi.org/10.1080/15567030802606038)
  21. Mengqi Z, Shi A, Ajmal M, Ye L, Awais M: Comprehensive review on agricultural waste utilization and high-temperature fermentation and composting. *Biomass Conversion and Biorefinery*. 2023, 13:5445-468. [10.1007/s13399-021-01438-5](https://doi.org/10.1007/s13399-021-01438-5)
  22. Zhu Q L, Wu B, Pisutpaisal N, et al.: Bioenergy from dairy manure: technologies, challenges and opportunities. *Science of the Total Environment*. 2021, 790:148199. [10.1016/j.scitotenv.2021.148199](https://doi.org/10.1016/j.scitotenv.2021.148199)
  23. Mignogna D, Ceci P, Cafaro C, Corazzi G, Avino P: Production of biogas and biomethane as renewable energy sources: a review. *Applied Sciences*. 2023, 13:10219. [10.3390/app131810219](https://doi.org/10.3390/app131810219)
  24. Kiyasudeen SK, Ibrahim MH, Quaik S, Ismail SA: An introduction to anaerobic digestion of organic wastes. *Prospects of Organic Waste Management and the Significance of Earthworms. Applied Environmental Science and Engineering for a Sustainable Future*. Springer, Cham; 2016. [10.1007/978-3-319-24708-3\\_2](https://doi.org/10.1007/978-3-319-24708-3_2)
  25. Rodríguez-Jiménez LM, Pérez-Vidal A, Torres-Lozada P: Research trends and strategies for the improvement of anaerobic digestion of food waste in psychrophilic temperatures conditions. *Heliyon*. 2022, 8:e11174. [10.1016/j.heliyon.2022.e11174](https://doi.org/10.1016/j.heliyon.2022.e11174)
  26. Menzel T, Neubauer P, Junne S: Role of microbial hydrolysis in anaerobic digestion. *Energies*. 2020, 13:5555. [10.3390/en13215555](https://doi.org/10.3390/en13215555)
  27. Christy PM, Gopinath LR, Divya D: A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. *Renewable and Sustainable Energy Reviews*. 2014, 34:167-73. [10.1016/j.rser.2014.05.010](https://doi.org/10.1016/j.rser.2014.05.010)
  28. Ohemeng-Ntiamoah J, Datta T: Perspectives on variabilities in biomethane potential test parameters and outcomes: A review of studies published between 2007 and 2018. *Science of The Total Environment*. 2019, 664:1052-62. [10.1016/j.scitotenv.2019.02.088](https://doi.org/10.1016/j.scitotenv.2019.02.088)
  29. Chinnici G, Selvaggi R, D'Amico M, Pecorino B: Assessment of the potential energy supply and biomethane from the anaerobic digestion of agro-food feedstocks in Sicily. *Renewable and Sustainable Energy Reviews*. 2018, 82:6-13. [10.1016/j.rser.2017.09.018](https://doi.org/10.1016/j.rser.2017.09.018)
  30. Xu S, Bi G, Liu X, et al.: Anaerobic Co-digestion of sugarcane leaves, cow dung and food waste: focus on methane yield and synergistic effects. *Fermentation*. 2022, 8:399. [10.3390/fermentation8080399](https://doi.org/10.3390/fermentation8080399)