# Biogas Production Potential from Cultivated Bulgarian Mushrooms

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***Abstract*— The annual mushroom consumption within the European Union surpasses 1060 thousand tons, with prices of fresh cultivated mushrooms steadily increasing, currently ranging from approximately 1.6 to 2.10 euros. Despite economic challenges, Bulgaria remains a leading producer of cultivated mushrooms in the Balkans, yielding nearly 11,000 tons annually. This study investigates the viability of using waste from cultivated Bulgarian mushrooms and dairy cattle manure as inoculum for biogas and methane production in laboratory anaerobic bioreactors. Homogenized mushroom waste was incubated for 30 days at 34.5 ± 0.05°C. The control sample showed the highest biogas yield at 4000 cm$^3$, while the sample with cultivated mushrooms exhibited the highest methane percentage of 53.07 % on the 14th day. The study highlights that using mushroom waste - cultivated mushrooms inhibits biogas production, potentially leading to reduced yields if incorporated into household waste used for biogas production.**

***Keywords*— *Biogas, Cultivated Bulgarian Mushrooms***

## I. INTRODUCTION

In recent decades, the issue of reducing the quantity of traditional energy sources has become increasingly relevant due to their rapid exploitation and potential environmental consequences. This necessitated the exploration of alternative environmentally friendly energy sources. The intersection of these concerns lies in the possibility of transforming organic waste into fuels, while simultaneously purifying them. This can be achieved through controlled anaerobic biodegradation of organic compounds, known as methanogenesis, resulting in the production of so-called biogas, with methane being its primary component.

Under natural conditions, methanogenesis occurs in the anaerobic zones of the Earth's biosphere, including various water bodies, lakes, rivers, mainly with muddy deposits, coastal marine sediments [1, 2], the digestive tracts of ruminant animals [3, 4, 5]. The same type of fermentation occurs in specially designed facilities for biogas production through the degradation of organic waste—manure, active sludge from aerobic treatment of domestic and industrial wastewater, and plant-based raw materials [6, 7]. The methane separated from these facilities can be directly utilized as an energy carrier, while the stabilized composted sludge can be used for fertilizing cultivated agricultural areas [6].

Due to the complex interactions among various types of microorganisms involved in it and the intricate transformations of the organic matter [8], methanogenesis is a highly unstable process. This necessitates the need for intensifying and stabilizing the process by introducing various stimulating substances into the medium that positively impact bacterial metabolism. Like any biochemical process, methanogenesis is challenging to control [9, 10] due to the inability to comprehensively influence the internal environment of the cells by manipulating their external surroundings in which they live. Various factors influence its progression and studying them would be of significant benefit.

The process of anaerobic biodegradation, which occurs in oxygen-depleted conditions and in the absence of sulfates and nitrates as electron acceptors, involves different associations of microorganisms and leads to the production of biogas (a gas mixture containing methane (50-70%), carbon dioxide (25-40%), and small amounts of hydrogen, nitrogen, and hydrogen sulfide). This process is commonly referred to as anaerobic biodegradation or methanogenesis [3, 11, 12].

Cultivated mushrooms represent a significant resource both as food and a commercial commodity. The cultivated mushroom (Agaricus bisporus) is an edible basidiomycete fungus belonging to the Agaricaceae family. It is cultivated in over 70 countries and is one of the most consumed mushrooms worldwide. Agaricus bisporus production in Bulgaria primarily takes place in four regions: Plovdiv-Pazardzhik, Haskovo, Rousse, and Varna. The overall mushroom production in the Plovdiv-Pazardzhik region is currently about 3,000 tons annually.

All of this underscores a significant opportunity to utilize mushroom waste (residues), with the aim of this article being to assess its impact on biogas and methane production during anaerobic fermentation with a manure mass from cattle. The evaluation utilizes parameters such as pH, dry matter, organic dry matter, methane content, and biogas production using a constructed laboratory setup and standardized analytical methods.

## II. MATERIALS AND METHODS

### A. *Biofermentation Setup*

For all experiments, a laboratory setup as depicted in Figure 1 was created. It consisted of the following components: a thermostatic water bath, a magnetic stirrer, two anaerobic bioreactors, and two gas collectors, with each of them connected to a gas analyzer that recorded the methane percentage content. The bioreactors used were hermetic and chemically inert polypropylene bottles with a volume of 400 cm$^3$, connected via silicone hoses and one-way gas valves to the biogas collectors with a volume of 2000 cm$^3$.

The biofermentation processes occurred in the aforementioned bioreactors, with the fermentation period lasting 30 days at a constant temperature of 34.5 ± 1°C and in an inert nitrogen atmosphere.

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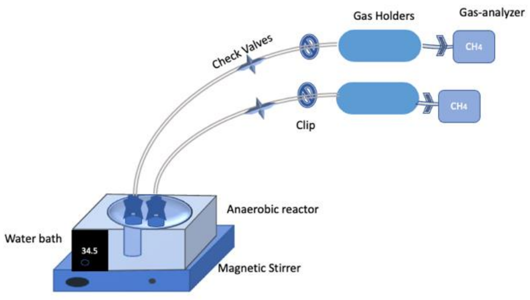

Fig. 1. Scheme of the laboratory installation.

The pH of the reference samples and mixtures was measured before and after biofermentation using a Hanna pH 211 instrument, with a range of 0 to 14 pH units and an accuracy of ± 0.01 pH, operating within a temperature range of 0 to 100 °C.

Every 48 or 72 hours, the total volumes of biogas accumulated in the gas collectors were measured using a graduated glass syringe for gas samples to determine the biogas volumes. Simultaneously, along with measuring the biogas volume, the gas was transferred to a holding gas container directed at measuring the relative methane content. For measuring the volume percentage (vol %.) of methane, a gas analyzer MS104K Diffusion Type with an infrared sensor was employed, operating in the range of -40 to +70°C for determining methane content in the biogas.

### *B. Preparation of the Biofermentation Mixtures*

The investigated mixtures were prepared using fresh biomass from cattle manure (Vrana Cattle Farm, Sofia, Bulgaria), which was sieved through a 0.5 mm mesh and vigorously stirred to obtain a homogeneous suspension [5]. The prepared reference samples (Sample I) contained equal amounts of pure cattle manure and distilled water, while the working mixture (Sample II) had the same composition, however, an 80% / 20% cattle manure / Agaricus bisporus mix was used.

The following parameters were determined: total solids (TS), moisture content, volatile solids (VS), and pH. These parameters were established both before and after anaerobic digestion processes. TS, VS, and moisture content of the three mixtures were determined using methods in accordance with BDS EN25934, EN15935, and BDS EN ISO 18134-1, respectively.

## III. RESULTS AND DISCUSSION

### *A. Analysis of the Reaction Mixtures Parameters*

A summary of the results for total solids (TS), volatile solids (VS) expressed as a percentage of TS, moisture content, and pH of the two reaction mixtures before the biofermentation process is presented in Table I. As observed from the results, the addition of 20% mushroom to the cattle manure feed led to an increase in its moisture content, resulting in a simultaneous decrease in TS due to the high water content of mushrooms.

TABLE I. PARAMETERS OF THE MIXTURES BEFORE ANAEROBIC DEGRADATION

| **Sample** | **Parameter** | | | |
|---|---|---|---|---|
| | ***Total Solids (TS, %)*** | ***Moisture Content (%)*** | ***Volatile Solids (VS/TS) %*** | ***pH*** |
| Sample I<br>Cattle Manure | 6.59 | 93.41 | 82.64 | 7.80 |
| Sample II<br>80% / 20%<br>Manure / Mushrooms | 4.31 | 95.70 | 94.44 | 8.00 |

Table II provides a summary of the same parameters obtained after the biofermentation process, with values recorded after the 30th day. In both cases, a reduction in TS and VS was observed, attributed to the conversion of organic matter into biogas during the anaerobic degradation process.

TABLE II. PARAMETERS OF THE MIXTURES AFTER ANAEROBIC DEGRADATION

| **Sample** | **Parameter** | | | |
|---|---|---|---|---|
| | ***Total Solids (TS, %)*** | ***Moisture Content (%)*** | ***Volatile Solids (VS/TS) %*** | ***pH*** |
| Sample I<br>Cattle Manure | 1.26 | 98.70 | 61.38 | 7.34 |
| Sample II<br>80% / 20%<br>Manure / Mushrooms | 3.46 | 96.54 | 72.33 | 7.31 |

### *B. Biogas Production and Methane Content*

The cumulative volume of biogas produced by the two reaction mixtures over the 30-day period is illustrated in Fig. 2.

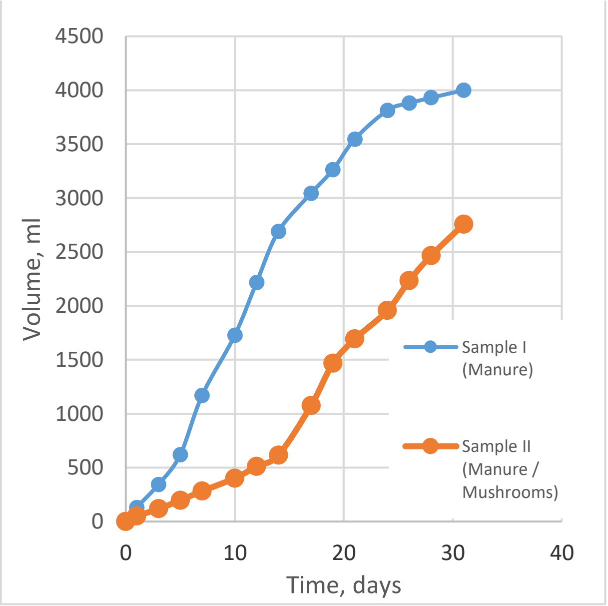

Fig. 2. Cumulative biogas volume released during the 30 days of anaerobic biofermentation.

Notably, the addition of mushroom mass to the cattle manure feed had an inhibitory effect on biogas production. By the end of the 30th day, the total biogas volume released by the reference sample (Sample I) was approximately 4000 mL, while in comparison, a total volume of 2750 mL was released by the 80%/20% cattle manure/mushroom mix (Sample II). It should be noted that, in the former case, the rate of biogas production evidently plateaued during the last week of the experiment, whereas a linear increase was observed when mushroom waste mass was added.

However, concerning the methane content of the biogas produced, surprisingly, the addition of mushroom mass had an observable positive effect, as demonstrated in Fig. 3. In the reference case (Sample I), the methane content steadily increased over the initial 10 days, peaking at 52 vol. %, followed by a sharp decline to approximately 11 vol. % by the 30th day. In contrast, while methane production remained relatively stagnant in Sample II, reaching a maximum of 10 vol. % during the initial 10 days, it steadily increased to 53 vol. % by the 14th day and maintained relatively stable values of 40 – 50 vol. % until the end of the 30-day experiment. This suggests that although the addition of mushroom mass to the anaerobic reaction feed increases the total moisture content, thereby negatively impacting the volatile solids (VS) available for methane generation, it overall has a positive effect on methane yield.

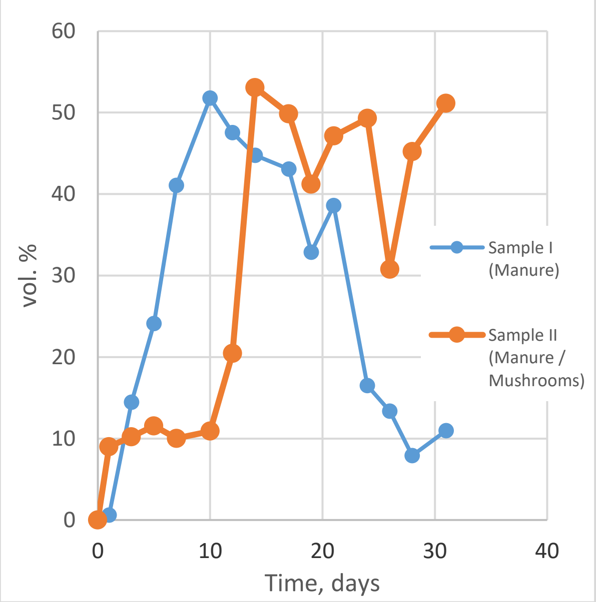

Fig. 3. Methane content of the biogas produced within the 30 days of anaerobic biodegradation.

The cumulative amount of methane produced in both cases (estimated by applying the methane volumetric content measurements data to the cumulative biogas production) is plotted in Fig. 4. Once again, it is noted that while lower yields are obtained from the reference sample in terms of methane content, the overall higher biogas production in this case led to increased yields. Nevertheless, it is confirmed that the higher methane content in the case of Sample II, coupled with the linear increase in biogas production throughout the last 15 days of the experiment, suggests that the mixture with the addition of mushroom waste (Sample II) shows more promise for larger long-term methane yields. Finally, although a comprehensive chemical analysis of the biogas produced by the two mixtures has not been conducted, the higher methane content of the biogas produced by Sample II possibly indicates that, in this case, the biogas could potentially contain lower levels of undesirable side products, such as hydrogen sulfide.

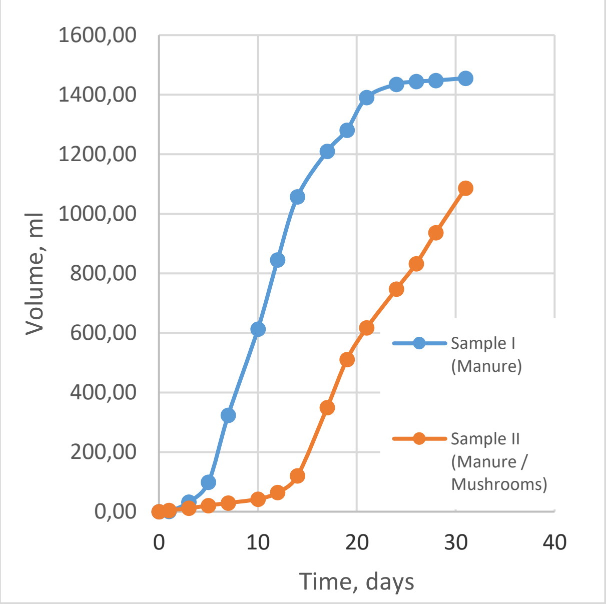

Fig. 4. Cumulative methane volume released during the 30 days of anaerobic biofermentation.

## IV. CONCLUSIONS

In summary, this study investigates the impact of incorporating cultivated mushrooms into cattle manure feed for the anaerobic biofermentation methane production process. The findings indicate that the inclusion of mushroom waste exerts an inhibitory influence on the total biogas production volumes. However, it concurrently results in elevated methane yields, as evidenced by the increased methane content within the biogas. This intriguing outcome suggests a promising avenue for optimizing methane production through the inclusion of mushroom waste in the anaerobic digestion process.

To further elucidate the underlying mechanisms responsible for this phenomenon, future research endeavors should delve into the specific chemical or biological agents present in the mushroom waste. A comprehensive understanding of these factors could lead to more efficient and sustainable biogas production processes, potentially reducing the occurrence of undesirable byproducts, such as hydrogen sulfide.

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