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Manufacturing A Bacterial Starter and A Digital Bio Fermenter to Treat Poultry Manure and Produce Methane Gas, CH_4

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Abstract: This study addressed the recycling of animal manure and the use of renewable energy sources through the safe and efficient application of organic fertilizers. The aim was to obtain and identify methane-producing bacteria and evaluate their effectiveness as biological starters in biogas production. Methanobacterium sp. was isolated from poultry manure, cow manure, and wastewater, and identified using morphological and biochemical methods. It was then cultivated for use as a starter in fermentation. Poultry manure was selected as the substrate, with four treatments applied: a control without starter, and three treatments with starters derived from poultry manure, cow manure, and wastewater. Results showed significant differences in bacterial abundance, with cow manure containing the highest count, at 11.52 Log (cfu/g), followed by wastewater, at 8.32 Log (cfu/g), and poultry manure, at 5.19 Log (cfu/g). Gas production indicators further confirmed that the cow manure starter (T3) achieved the highest methane yield, reaching 166 ppm on day 11, followed by the wastewater starter (T4, 72 ppm), the poultry starter (T2, 110 ppm), and the control (50 ppm). Based on these findings, a digital biofermenter (T5) was developed and tested, demonstrating superior efficiency by producing 185 ppm on day 9, which was 65 ppm higher than T3 at the same time and two days earlier. In conclusion, cow manure was identified as the most effective source of methanogenic bacteria, and the developed biofermenter significantly improved both gas yield and fermentation time, providing a practical innovation for clean energy production and environmental sustainability.

Keywords: Anaerobic digestion, Arduino Nano project, Bioconversion, Climate, Environment, Waste valorization.

Introduction

Commercial poultry production has expanded rapidly in recent decades in response to the growing global demand for meat and eggs [1, 2]. This rapid intensification has inevitably led to the generation of large quantities of poultry manure, posing significant environmental challenges. Among these challenges are water and soil contamination, the emission of unpleasant odors, and the release of greenhouse gases, all of which contribute to environmental degradation and public health concerns [3]. These pressing issues underscore the critical need for sustainable management strategies that not only mitigate pollution but also enable the recovery and utilization of valuable resources contained in poultry waste.

In this context, anaerobic digestion has emerged as a promising approach for managing poultry manure. This widely studied biological process converts organic matter into biogas, predominantly composed of methane (CH_4) [4, 5, and 6]. However, the efficiency of poultry manure as a substrate for anaerobic digestion is often limited by its relatively low carbon-to-nitrogen (C/N) ratio compared with other livestock manures [7]. To address these limitations, researchers have explored various strategies to improve substrate biodegradability and biogas yield. These strategies include co-digestion with other organic materials [8, 9, and 10], mechanical or chemical pretreatments to improve substrate accessibility [11, 12], and the addition of microbial inoculants to stimulate and stabilize fermentation processes [5, 13].

Moreover, recent studies have shown that combining poultry manure with complementary substrates such as algae or food waste can further increase methane production and overall process efficiency [13, 14, and 15]. These findings highlight the considerable potential of poultry manure as a renewable energy feedstock while emphasizing the importance of developing cost-effective and scalable approaches that optimize both environmental and economic outcomes.

Despite these advances, several gaps remain in the current literature. Notably, there is limited research on specialized

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microbial starters capable of accelerating fermentation and maintaining stable methane production. Although microbial diversity and its role in anaerobic digestion have been described [7, 8], few studies have systematically compared microbial starters derived from different biological sources under standardized conditions. Additionally, many experimental systems still lack affordable, practical tools for real-time monitoring of gas production, which restricts the optimization of process performance and operational efficiency.

To address these challenges, the present study aims to develop an integrated biological and technological framework for the effective utilization of poultry manure. This involves isolating and characterizing methanogenic bacteria from various sources to produce high-performance microbial starters, as well as designing a digital biofermentor equipped with real-time monitoring capabilities. By combining microbial technology with digital monitoring, this approach is expected to enhance methane yields, reduce environmental impacts, and provide a sustainable, low-cost model for waste management and renewable energy generation. Ultimately, such advancements may contribute significantly to the development of sustainable recycling strategies for poultry waste.

Materials and Methods

Manure types and preparations

Poultry manure, cow manure, and wastewater samples were collected from farms affiliated with the University of Basrah, Basrah Governorate, in southern Iraq. Fresh samples (<24 hours) were aseptically collected in sterile 500 mL polypropylene containers. Samples were transported on ice at 4 °C to the laboratory and stored at 4 °C until analysis, ensuring processing within 24–48 hours.

The initial pH of the substrates was measured and adjusted to the optimal range for methanogens, between 6.8 and 7.2. Fermentations were carried out under mesophilic conditions at 35 ± 2 °C. Total solids (TS) and volatile solids (VS) were determined according to the standard methods of the American Public Health Association [16]. The carbon-to-nitrogen (C/N) ratio of poultry manure was maintained at approximately 25:1, which is considered optimal for anaerobic digestion.

Isolation of bacteria

Methanobacterium sp. was isolated from three sources: poultry manure, cow manure, and wastewater. Colonies were purified through three successive subcultures and maintained on thioglycollate agar medium (LAB M Limited, Heywood, England, UK) under anaerobic conditions at 37 °C. Gram's stain kit (Titan Biotech Ltd., Rajasthan, India) was used for staining.

Identification and diagnosis of the bacterial isolates were based on morphological, microscopic, and biochemical characteristics. Phenotypic observations included colony shape and Gram staining, while biochemical tests comprised catalase, indole, starch hydrolysis, casein hydrolysis, gelatinase, carbohydrate fermentation, methyl red, Voges–Proskauer, and citrate utilization. The results were compared with Bergey's Manual of Systematic Bacteriology to determine the genus and characteristics of the isolated methanogenic bacteria [17].

Although these classical methods allowed preliminary identification, it is recommended that future studies apply molecular techniques, such as DNA isolation and 16S rRNA sequencing, to achieve higher taxonomic accuracy and confirm the identity of the isolates.

Experimental design and replicate.

The experiment was conducted using poultry manure as the substrate with four treatments in triplicate (n = 3):-

T1 (control): Poultry manure without bacterial starter (1000 g only).

T2: Poultry manure + 10 mL starter isolated from poultry manure per 1000 g.

T3: Poultry manure + 10 mL starter isolated from cow manure per 1000 g.

T4: Poultry manure + 10 mL starter isolated from wastewater per 1000 g.

Devices and bioreactors

A digital bio-fermenter was locally designed and assembled using spare parts readily available in the local market at low cost. The system consisted of two main components:

1. Electronic system: The monitoring unit was fabricated and calibrated in accordance with the standards of the Iraqi Ministry of Environment, Thi Qar Environment Directorate. It was tested repeatedly on separate samples, demonstrating reliable performance as later detailed in the Results and Discussion section. The unit integrated an Arduino Nano microcontroller, an MQ-series methane (CH_4) gas sensor, an LCD screen, and a Wi-Fi communication module. For accuracy and reproducibility, the sensors were calibrated using certified methane reference gases at concentrations of (0, 2, and 5) % before practical operation. The reliability of the device was further confirmed through comparison with reference measurements conducted by mobile air-quality monitoring stations operated by the Iraqi Ministry of Environment and the Thi Qar Environment Directorate, which are specifically equipped for assessing gaseous emissions. Figure 1 presents the main components of the device, while Figure 2 illustrates the calibration process and the comparison with the Ministry's mobile monitoring vehicle.

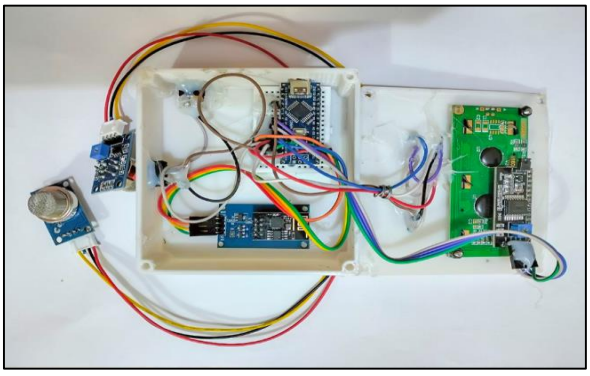

Figure (1): The electronic parts that make up the digital bio-fermenter device.

Figure (2): Calibration method and comparison of results with the vehicle designated for measuring gases.

Measuring methane gas (CH4)

After preparation, the samples were placed in sealed plastic bottles connected to MQ-series methane (CH_4) sensors, which were interfaced with Arduino boards. These electronic sensors, equipped with electrodes for methane detection, had been pre-calibrated in accordance with the standards of the Iraqi Ministry of Environment and the Thi Qar Environment Directorate. During the measurement process, the electrodes of each sample were connected sequentially to the Arduino system, which was programmed to transmit the recorded data directly to a computer. This configuration enabled real-time monitoring and accurate quantification of methane production across the different samples. Figure 3 illustrates the experimental setup used for measuring methane gas.

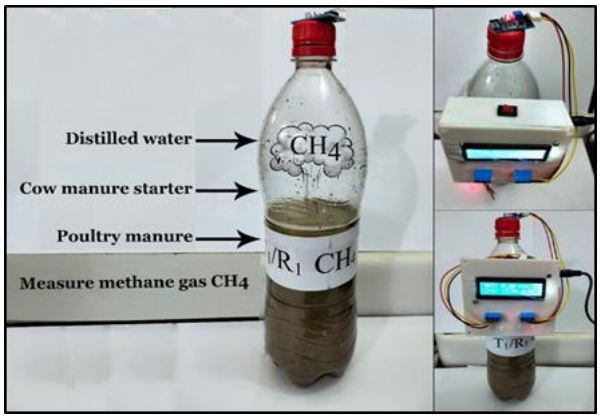

Figure (3): Method for measuring methane gas for different samples.

2. Fermentation vessel: A cylindrical steel vessel with a capacity of 13.5 kg and a pressure tolerance of 260 psi was employed as the fermentation unit. The vessel was fitted with dedicated inlets for substrate feeding and outlets for post-fermentation discharge. To ensure controlled conditions, it was equipped with a thermostat for temperature regulation and a methane sensor for continuous monitoring of gas production. The generated data were transmitted wirelessly via the integrated Wi-Fi module, allowing remote and real-time monitoring through a mobile phone application. Figure 4 shows the details of the manufactured digital biofermentor.

Figure (4): Details of the manufactured digital bio fermenter.

Statistical analysis

All treatments were carried out in triplicate (n = 3). Statistical analysis was performed using SPSS (version 2018) [18]. Data were tested for normality using the Shapiro–Wilk and for homogeneity of variances using Levene's test. A one-way ANOVA was applied, followed by Duncan's multiple range test at a significance level of $p < 0.05$, to determine significant differences among treatments.

Results and discussion

Results of the identification of methanogenic bacteria

The morphological examinations included the description of the colonies in terms of shape, surface, edge, color, and size. The colonies appeared spherical, with a raised surface, flat edges, white coloration, and a small diameter. Gram staining results showed that the methanogenic colonies were Gram-negative and exhibited a cell wall structure rich in lipids.

Table (1) presents the results of the biochemical tests for the methanobacterial colony. The morphological, microscopic, and biochemical characteristics were entirely consistent with the descriptions provided in Bergey's Manual of Systematic Bacteriology [17].

Table (1): Results of biochemical tests for the *methanobacterial* colony.

NO.	Biochemical Tests	Test results
1	Catalase Test	-
2	Indole Test	+
3	Starch Hydrolysis	+
4	Hydrolysis of Casein	+
5	Gelatinase Hydrolysis	+
6	Carbohydrate Fermentation	+
7	Red Instance Test	+
8	Fox-Proskauer Test	-
9	Test citrate	+

+ indicates a positive result.
- indicates a negative result.

The results of the statistical analysis of microbial counting operations in different samples in Figure (5) show that there are significant differences ($P \leq 0.05$) between the studied samples, represented by recording the highest content of methane-producing bacteria in the cow manure sample, which was in first place at 11.52 Log (cfu/g), and the wastewater sample, which was in second place at 8.32 Log (cfu/g). The poultry manure sample ranked third or last in terms of the abundance of its methane-producing bacteria, with an amount of 5.19 Log (cfu/g).

Based on what was shown by the Duncan test in the statistical analysis of the various samples, we can prove statistically that the cow manure sample is the most active or effective in terms of containing the most significant number of methane-producing bacteria, and that this procedure is considered the first step; To identify the highly effective active starter in the manure fermentation process and raise its economic efficiency in analyzing its raw materials and producing methane gas.

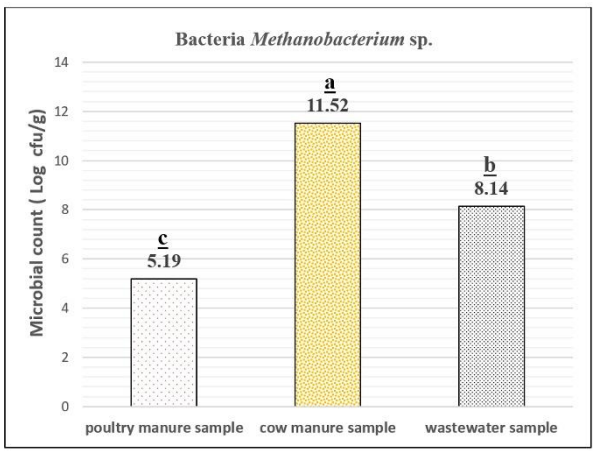

Figure (5): Microbial counting results for *Methanobacterium* sp. Isolated from three different sources.

Evaluation of starters for methane production

We are not satisfied with the first step of determining the optimal starter in the fermentation and gas production process.

There is a second, more effective step, through which the most efficient starter in gas production will be determined by experimenting with the starters mentioned earlier and assessing their efficiency in production. This will become clear from the gas production indicators and comparison between them in Figure (6), which shows the CH4 methane emission indicators during 14 days for four experimental treatments. It is clear from the figure that the highest gas production indicator, at 166 ppm, was recorded by the third treatment, T3, on day 11 of the fermentation process. In first place and in a very record time compared to the other treatments, the fourth treatment, T4, came after it, recording a rate of 72 ppm during day 10 of the fermentation process, while the second treatment, T2, recorded a rate of 110 ppm at the end of the experiment, which amounted to 14 days. Finally, the empty control treatment came. From the addition of the starter, the lowest value recorded in the indicator was 50 ppm at the end of the experiment. This indicator is considered unsatisfactory in terms of both the gas level and the speed of its production for the first and second treatments, respectively.

We notice that if the same starter and the amount of material used in the third treatment were used, which gave the best results, it was placed (starter + the amount used in treatment T3) in the bio fermenter that was manufactured recently, under the name of the T5; As an effective indicator to indicate its efficiency in terms of the speed of fermentation and the level of gas produced, as shown in Figure (7), two indicators of gas production using the manufactured bio fermenter and the other method that was used in this scientific experiment in treatment T3.

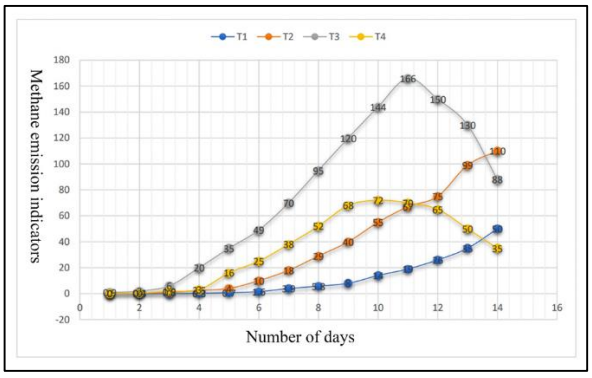

Figure (6): Methane emission indicators over 14 days for four experimental treatments.

It is clear from the two indicators in the third treatment, T3, and T5, that the biogas production index of the fifth treatment (the bio fermenter treatment) was significantly superior in terms of the gas level and fermentation time, as the bio fermenter treatment recorded the highest level of fermentation on the ninth day of production, at a rate of 185 ppm compared to the third treatment in the previous experimental method, which recorded 120 ppm at the same time (the data for the third treatment in Figure (6) were quoted and transferred to Figure (7); for comparison and evaluation of the efficiency of the manufactured fermenter.

Therefore, the time difference can be used to infer the speed of fermentation and the maximum rate of production through Figure (7). The highest rate recorded for the third treatment was on day 11 of the fermentation process, while the highest rate was recorded for the T5 on day 9. That is, there is a 48-hour time difference.

This is considered critical evidence of the efficiency of the manufactured biofermentor in shortening the fermentation time, as evidenced by the rate recorded on the ninth day, which reached 185 ppm, the highest indicator. Suppose it were compared to the highest rate during the same time (the ninth day), which reached 120 ppm. In that case, we will obtain a difference in the level of gas production of 65 ppm. Thus, we can evaluate the efficiency of the manufactured fermenter in terms of its speed and fermentation efficiency, as well as its digital screen and mobile phone application for monitoring gas levels and fermentation time.

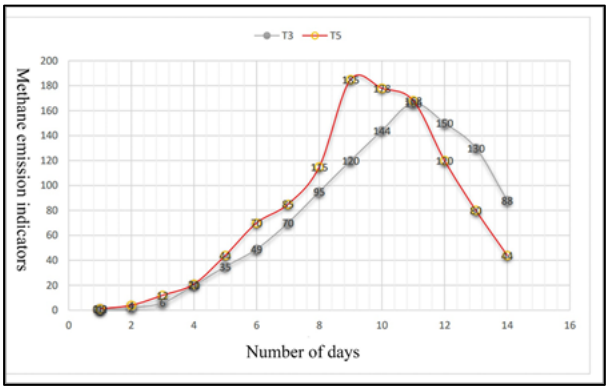

Figure (7): Methane gas emission indicators over a period of 14 days for the biofermenter and the previous experimental.

Method

Developing a method for reading samples through digital electronic imaging and transforming it into a fully integrated device represents a significant advancement in fermentation monitoring and gas measurement. The device was tested using the procedures described above, and the results confirmed its efficiency in the fermentation process and the accuracy of gas quantification. This innovation is particularly valuable for monitoring methane (CH_4) emissions from livestock facilities, enabling precise and individualized measurements across various experimental treatments. Methane is recognized as the second most influential greenhouse gas after carbon dioxide, contributing substantially to global warming and ground-level ozone pollution.

Beyond its technical contribution, this method has significant environmental and industrial implications. Promoting the controlled collection of animal manure and utilizing the resulting biogas for industrial and commercial purposes helps prevent uncontrolled emissions into the atmosphere, thereby mitigating climate change.

From a technical perspective, the developed device functions as a compact laboratory, integrating both fermentation and measurement processes. Unlike conventional methods, it does not require separate equipment such as an incubator to maintain optimal fermentation temperatures or additional gas analyzers, which are often costly and unavailable in many laboratories. Traditional gas monitoring in Iraq, for instance, relies on mobile vehicles of the Ministry of Environment to measure atmospheric gases. Such systems do not provide precise insights into the specific contribution of each sample to methane production.

When compared with earlier techniques, the advantages of the new device become more evident. Previous researchers [19] employed the water displacement method, in which a gas outlet tube is inserted into an inverted graduated cylinder filled with a 28% saline solution. The salt solution prevents gas dissolution, and the displaced volume represents the gas produced. While this method has been widely adopted, it requires multiple

accessories and considerable handling. In contrast, the newly developed device simplifies the process, provides accurate digital readings, and eliminates the need for additional components, offering a practical alternative for researchers.

The economic dimension of this innovation is also noteworthy, particularly in relation to poultry manure. Poultry waste typically exhibits low gas productivity compared to cattle manure, owing to its low carbon-to-nitrogen ratio [7]. To address this limitation, a biological starter derived from cow manure was introduced to enhance biodegradation efficiency and methane yield. The methanogenic bacteria present in cow manure facilitated the construction of carbon structures and supported active methane generation.

Furthermore, the device significantly reduced the fermentation period. This improvement was attributed to both the use of an effective starter and the controlled conditions provided by the built-in thermostat. Results were displayed on the device's digital screen and synchronized with a mobile application, ensuring real-time monitoring. Compared with previous studies [20], where methane production required up to 23 days, the current system achieved comparable yields in a maximum of 14 days, as illustrated in Figure (5).

Limitations: Factors such as pH fluctuations, substrate composition, microbial community variability, and potential accumulation of inhibitory compounds may influence the efficiency and reproducibility of methane production.

Conclusion

From the above, we conclude that the cow manure sample is the most active because it contains the most significant number of bacteria that produce methane gas (CH4).

It was also observed that there was a significant increase ($P \leq 0.05$) in the biogas production index of the T5 (the bio fermenter treatment), in terms of the gas level and fermentation time, as the bio fermenter treatment recorded the highest level of fermentation on the 9th day of production, with a rate of 185. Ppm compared to the third treatment, which recorded 120 ppm at the same time. This is considered one of the critical pieces of evidence in evaluating the efficiency of the manufactured biofermentor, which contributed to a significant shortening of the fermentation time. This is a result of the activity of the added starter and the device's efficiency in meeting the requirements for the fermentation process.

Disclosure statement

- **Ethics approval and consent to participate:**

  The study was approved by the Scientific Committee of the Department of Animal Production, College of Agriculture and Marshes, University of Thi-Qar. All procedures were conducted in accordance with national and international guidelines for animal care, and ethical approval was granted under No. AP.21 on 15 January 2025.
- **Author's contribution:** The researchers confirm their contribution to the research paper as follows: Preparation of the research plan, statistical analysis, modeling, and instrumentation design: Dr. Ahmed Al-Salhi; biological tests, agricultural media, and collection of results: Dr. Sabah Al-Shatty and Dr. Iman Al-Amara; review of the research, preparation of technical and technological requirements: Ms. Afrah Al-Rikabi.
- **Conflicts of interest:** The authors declare that there is no conflict of interest regarding the publication of this article.
- **Consent for publication:** Not applicable.
- **Availability of data and materials:** His raw data required to reproduce these findings are available in the body and illustrations of this manuscript.
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